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ABBREVIATIONS OF TITLES

ABBREVIATION	NAME OF PERIODICAL
Agric. J. India	Agricultural Journal of India.
Agric. Res. Inst. Pusa	Agricultural Research Institute, Pusa.
Amer. J. Pub. Health	American Journal of Public Health.
Anal. Fis. Quim.	Anales de la Sociedad Española Física y Química.
Annalen	Justus Liebig's Annalen der Chemie.
Ann. Appl. Biol.	Annals of Applied Biology.
Ann. Chim. Applicata	Annali di Chimica Applicata.
Arch. Pharm.	Archiv der Pharmazie.
Ber.	Berichte der deutschen chemischen Gesellschaft.
Biochem. J.	The Biochemical Journal.
Biochem. Zeit.	Biochemische Zeitschrift.
Bull. Imp. Inst.	Bulletin of the Imperial Institute.
Centralbl. Bakt.	Zentralblatt für Bakteriologie.
Chem. Ind.	Chemistry and Industry.
Compt. rend.	Comptes rendus hebdomadaires des Scéances de l'Academie des Sciences.
D. R. P.	Deutsches Reichspatent.
Eng. News Rec.	Engineering News Record.
Florida Agric. Expt. Sta. Bull.	Florida Agricultural Experimental Station Bulletin.
Helv. Chim. Acta	Helvetica Chimica Acta.
Ind. Eng. Chem.	Industrial and Engineering Chemistry.
Ind. J. Med. Res.	Indian Journal of Medical Research.
Ind. Sci. Congress, Med. Sec.	Indian Science Congress, Medical Section.
International Cong. App. Chem.	International Congress of Applied Chemistry.
J. Agric. Sci.	Journal of Agricultural Science.
J. Amer. Chem. Soc.	Journal of the American Chemical Society.
J. Amer. Soc. Agron.	Journal of the American Society of Agronomy.
J. Biol. Chem.	Journal of Biological Chemistry.
J. C. S.	Journal of the Chemical Society.
J. Gen. Physiol.	Journal of General Physiology.
J. Ind. Chem. Soc.	Journal of the Indian Chemical Society.
J. Ind. Inst. Sci.	Journal of the Indian Institute of Science.
J. Int. Soc. L. T. C.	Journal of the International Society of Leather Trades Chemists.
J. Min. Agric.	Journal of the Ministry of Agriculture and Fisheries.
J. Pharm. Soc. Japan	Journal of the Pharmaceutical Society of Japan.

Abbreviation	Name of Periodical
J. Phys. Chem. ..	.. Journal of Physical Chemistry.
J. Physiol. ..	.. Journal of Physiology.
J. pr. Chem. ..	.. Journal für praktische Chemie.
J. S. C. I.	.. Journal of the Society of Chemical Industry.
J. S. C. I., Rev. ..	.. Journal of the Society of Chemical Industry, Review.
J. Soc. Chem. Ind. ..	.. Journal of the Society of Chemical Industry.
J. West. Soc. Eng. ..	.. Journal of the Western Society of Engineers.
Monatsh.	.. Monatshefte für Chemie und verwandte Theile anderer Wissenschaften.
Perf. and Essent. Oil Rec.	.. Perfumery and Essential Oil Record.
Proc. Ind. Sci. Cong.	.. Proceedings of the Indian Science Congress.
Proc. Roy. Soc. ..	.. Proceedings of the Royal Society.
Proc. Roy. Soc. Canada	.. Proceedings of the Royal Society of Canada.
Soil Sci.	.. Soil Science.
Zentralbl. Bakt. ..	.. Zentralblatt für Bakteriologie.
Z. pflanz. D. ..	.. Zeitschrift für Pflanzenernährung und Düngung.
Z. physikal. Chem. ..	.. Zeitschrift für physikalische Chemie, Stöchiometrie und Verwandtschaftslehre.
Z. physiol. Chem. ..	.. Hoppe-Seyler's Zeitschrift für physiologische Chemie.

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SALT STAINS ON SOUTH INDIAN HIDES AND SKINS.

By V. N. Patwardhan and M. Subramania Sastry.

The term *salt stain* has been applied to defects in hides or skins which arise during the curing period. Stather (*Collegium*, 1928, 567) groups these defects under three heads: (1) those in which curing salt does not play any part, *e.g.*, red heat and blue and purple colorations, which are due to the pigments produced by bacteria growing on the hide substance (Lloyd, *J. Int. Soc. L. T. C.*, 1929, **13**, 538; Bergmann, *ibid.*, 1929, **13**, 599; Stather and Liebscher, *Collegium*, 1930, 151, 170), (2) defects produced mechanically by the action of salt crystals within the hide, or on its surface and (3) true salt stains. The last are spots caused by impurities in the curing salt, or the denaturant added to it.

According to Stather, there are two kinds of salt stains, (*a*) pale yellow to orange scale-like specks on the flesh side, measuring 3–5 mm. in diameter (Becker, *Collegium*, 1912, 408) and (*b*) lemon-yellow to orange-brown stains of diameter 4–8 mm. (Becker, *loc. cit.*; Abt, *Collegium*, 1912, 388; 1913, 204). Becker succeeded in producing yellow or orange stains by using pure cultures of bacteria isolated from the corresponding stains. He was not able to produce blue stains by mere bacterial action, however, and concluded that these might be due to chemical changes not involving bacterial activity. Abt maintained that micro-organisms are only secondary in stain-formation; he found the stained regions always gave more intense qualitative tests for iron than the unstained. According to him, calcium sulphate in the curing salt is precipitated as calcium phosphate by reaction with ammonium phosphate derived from the nucleic acid of the skin. The resulting ammonium sulphate reacts with insoluble ferrous carbonate forming soluble ferrous sulphate which forms a stain by combining with skin protein. He recommended adding small quantities of sodium carbonate to the curing salts, since the former precipitates calcium sulphate and thus prevents further changes.

The peculiar type of stains with which the present investigation deals belongs to the third type described by Stather. These stains occur chiefly on the wet salted hides coming from the Malayalam districts, a tract parallel to the western sea-coast of the South Indian Peninsula. The colour of the stains ranges from blue-green to violet, and is not seen either in the cure, or in the soaks, developing only when the stained hides and skins are soaked in the lime liquor. Thus it will be seen that the tanners purchasing hides and skins from these areas suffer a loss owing to the absence of any criterion by which they could distinguish the stained from the unstained goods at the time of buying. There is no hair-slipping accompanying these stains as is generally the case with

stains described by previous workers. The colour of the stain is greenish blue, deep blue, or bluish purple in the lime liquors, but changes to black as the vegetable tannage proceeds, remaining as black spots on the finished and dressed hides. The defective hides are useless for light colouring and, in consequence depreciate in value.

The various problems arising out of this investigation have been taken in the following order:—(1) A study of the curing conditions in Malabar, (2) the nature of the micro-organisms connected with stain production, (3) chemical examination of stained and unstained hides and of curing salts, (4) experimental production of stains, (5) treatment for the removal of stains and (6) prevention of stain-formation.

Curing Conditions in Malabar.

Large curing centres in Malabar are situated in towns which are far apart. The hides and skins are collected in small numbers at a time by villagers who cure them with a very low grade of salt, generally mixed with earth to increase their weight. The goods are bought by small collectors who sell them to big merchants in towns, where they are salted and stored in godowns till the accumulated thousands are despatched wet-salted to various tanning centres in South India.

A study of the conditions prevailing in the godowns throws considerable light on the problem in hand. The godowns are generally very dirty, badly ventilated and unprovided with adequate drainage. The hides are not washed, but are directly rubbed with a low grade of sea-salt which is often mixed with salt previously used for curing; they are then folded and piled on the floor or over a cement platform a foot from the floor. Generally two to three days elapse between the purchase of the hides and their salting and storage in godowns, during which period they are saved from partial decomposition by the crude cure originally given by the villagers. After the first salting in the godown, the hides are left in piles for about a fortnight, while the brine formed by abstraction of water from the hide percolates through the pile and collects on the floor. In many cases the volume of brine thus formed is so great that it actually rises above the platform and covers the lower layers of the pile. After fifteen days a second salting is given, the hides being refolded and repiled; in this condition they remain in the godowns for more than six weeks before despatch to the tanning centres.

The climatic conditions in Malabar are peculiar to those prevailing on the south-western coast of India with a high average rainfall, for eight months the atmosphere being practically saturated with moisture. Reports from the tanners point out that the highest percentage of stained hides, which sometimes reaches as much as 30 per cent., occurs in the lots despatched from Malabar during the second half of October and the whole of November. After that period the percentage of stained hides decreases till it reaches a minimum at the beginning of the monsoon in June. Although the tanners do not maintain detailed statistics, the information is based on years of experience in the Malabar hide trade, so that the figures cited above can be safely assumed to be very nearly correct. A comparison with data for the rainfall of Malabar

will reveal the fact that hides accumulated during the south-west monsoon, which extends from June to September or October, show a great tendency to become stained; thus during the wet season there is increased incidence of stains.

In this connection it should be noted that the goat or sheep skins coming from the Malayalam districts contain a much lower stained percentage than hides from the same area. The reason for this difference may lie in the fact that the skins are carefully salted with greater cleanliness, and are not generally allowed to accumulate in the godowns over so long a period as the hides. The skins fetch comparatively higher prices than the hides and hence the extra care bestowed on them.

The brine accumulated on the floor is very offensive in odour, and contains much decomposing and putrefying organic matter; it is rich in hydrogen sulphide and saprophytic organisms, and is drained away only when the collected hides are despatched and the next collection begins. The atmosphere of the godown is saturated with moisture.

In some places the hides are kept immersed in saturated brine in large cement-lined pits, such brine being generally very dirty. The hides are allowed to accumulate in the pits till favourable market conditions warrant their being sold. When fresh hides are introduced the old brine is removed, but more dirty salt is thrown in to make up for the dilution.

Micro-organisms in curing salts and on hides and skins.

Stained portions of the mechanically delimed hides were introduced into sterile nutrient broth and the more prominent organisms then developing isolated by the usual methods. A number of such micro-organisms have been systematically studied and will be described in detail elsewhere. Generally speaking they are of two types, those occurring in the soil and those associated with the skins of animals.

Attempts were made to induce stain formation by smearing thoroughly washed pieces of skins of freshly slaughtered sheep with pure or mixed cultures of isolated bacteria, followed by rubbing with good quality common salt for preservation. In all the cases the results were negative, indicating that none of these bacteria was specific in producing the characteristic stains, although they may be an important factor, as will be explained later. Halophilic bacteria of the pigment-producing type were also isolated; they produced red or orange pigments, and may resemble those described by Lloyd (*loc. cit.*), but their study was not pursued since they could not be responsible for producing blue, purple or violet stains on hides.

Analyses of stained and unstained hide portions and of curing salts.

(*a*) *Curing salts.*—Table I shows the percentage of the more important constituents of three samples of salt. Khari salt consists mainly of sodium sulphate and is exclusively used in the provinces of Bengal and Behar for salting hides. Ernakulam and Trivandrum salts are locally manufactured along the sea-coast; these with others prepared at individual centres are used in Malabar for curing.

TABLE I.

Constituent	Khari		Ernakulam		Trivandrum	
	Water soluble	Soluble in conc. HCl	Water soluble	Soluble in conc. HCl	Water soluble	Soluble in conc. HCl
Na	19.21	..	35.47	..	32.41	..
Mg	2.358	0.772	1.074	0.127	0.440	0.163
Ca	0.207	2.251	0.229	0.236	0.136	0.300
Fe	0.003	0.08	..	0.032	..	..
Cl	5.697	..	54.71	..	49.98	..
SO_4	36.42	..	1.44	..	1.608	..
Insoluble	..	..	..	1.33	..	2.66

(*b*) *Stained and unstained leather portions.*—The material was taken from vegetable tanned and dressed hides in local tanneries. The hides were examined, stained portions removed, and then cut or chopped as fine as possible and mixed thoroughly. Unstained portions from the stained hides were also treated in the same manner. The materials were then ignited and the resulting ash analysed, typical results being given in table II.

TABLE II.

Sample	Fe	Ca	Mg
Stained	0.024	0.0752	0.0088
Unstained ..	0.017	0.0859	0.0054
Stained	0.0579	0.0545	0.0134
Unstained ..	0.0358	0.0550	0.0104
Stained	0.0542	0.0487	0.0182
Unstained ..	0.0394	0.0787	0.0132

From the above it will be clear that iron is invariably higher in stained than unstained portions; magnesium shows a similar variation, but the differences in calcium are inconsistent. The quantity of calcium in any part of the skin will depend upon the thoroughness of deliming, which in South Indian tanneries is effected mechanically by repeated scudding and washing with water. The higher percentage of iron in the stained portion has a significance which will be seen from later observations.

Experimental production of stains.

In Malabar, the salt generally used for curing is a mixture of used and unused salts, the used salt commonly containing a large quantity of earth, and some organic matter derived from the hide. The quantity of sodium chloride

in the used salt is very much less than in the fresh, and, consequently, when a mixture is applied there is always danger of there being less salt than is required for effective curing. Moreover, the used salts may contain several types of micro-organisms forming a heavy inoculum and thus leading to rapid stain-formation. To determine whether such was really the case, experiments were conducted with different mixtures of fresh and used salts, and of these with soil. Skin pieces were cured with (1) best grade of bazaar common salt, (2) a mixture containing equal weights of used and fresh salts, (3) a mixture of soil with the best grade bazaar common salt, and (4) a mixture of soil and used salt. The pieces thus treated were kept moist for over two months before examination, when it was observed that none had developed any stain, either in the soaks, or after liming and de-hairing.

In another series of experiments the skin pieces were preserved by immersion in brine instead of rubbing with salt, the cures being made up from best grade bazaar common salt, used salt and soil. The brine was saturated initially, and the skins were kept in the cure for six weeks before examination. Results are given in table III.

TABLE III.

Cure	P_H	Stain
Fresh salt	7.2	Nil.
Fresh salt+soil ..	7.2	"
Used salt	6.6	"
Used salt+soil ..	6.6	Positive.
Used salt, sterilised	6.4	Nil.

The stains on pieces cured with used salt mixed with soil resembled the natural stains in colour. This observation together with the previous one that a greater amount of iron is present in stained portions than in the corresponding unstained ones led to the inference that iron was one of the constituents of the stains, and that it may have been derived from either the curing salt or the soil.

To determine whether iron only was responsible for the stain-formation, the following experiment was made. The brine (30 and 25 per cent.) was prepared from pure sodium chloride, and 500 c.c. of brine was used for each bottle, to which ferrous sulphate (0.5 per cent.), calcium chloride (0.5 per cent.) and magnesium chloride (0.5 per cent.) were added in different combinations. Skins from freshly slaughtered goats were washed thoroughly, cut in pieces of 2″×3″ or 2″×4″ and kept immersed in these solutions for about four months, when they were examined. Not one showed stains in the soaks, but after liming for three days, the stains came up in all cases where iron was present. De-hairing showed the patterns to be strikingly identical with those obtained in nature.

TABLE IV.

Cure	Stain
NaCl alone	Nil.
$NaCl+FeSO_4$	Positive.
$NaCl+FeSO_4+CaCl_2$	”
$NaCl+FeSO_4+CaCl_2+MgCl_2$ ..	”
$NaCl+CaCl_2$	Nil.
$NaCl+CaCl_2+MgCl_2$	”
$NaCl+MgCl_2$	”
$NaCl+MgCl_2+FeSO_4$	Positive.

From the above table it will be obvious that calcium and magnesium salts have no influence on stain-formation, and that when iron salts are present in the cure the stains develop even in absence of added calcium or magnesium salts. It may further be adduced that iron which might be present on the skin pieces as hæmoglobin from unwashed blood is not sufficient for stain-production.

The accompanying photographs show the appearance of the artificial as well as the natural stains.

In a fourth series of experiments the pieces were soaked in different cures containing either iron salts or specimens of laterite soil together with sodium chloride (30 per cent.) and examined for stains in the manner described already.

TABLE V.

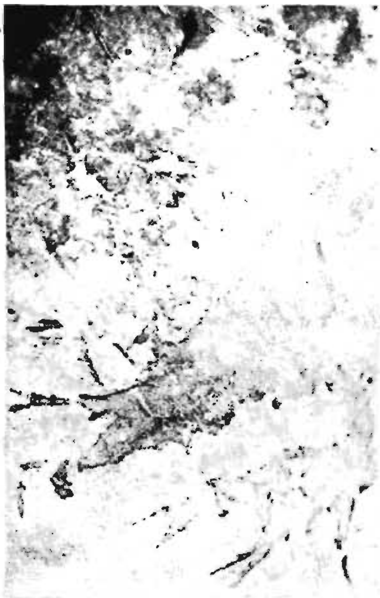
Brine with	Stains
Laterite soil	+
Laterite+NaF	+
$FeSO_4$	+++
Fe_2O_3	+
$FeSO_4$+water extract of laterite soil ..	++
Fe_2O_3+ ” ” ..	+

+, visible ; ++, fairly heavy ; +++, very intense.

Discussion.

That iron salts are responsible for the stain-production has been amply proved by the curing experiments described in the last section. An attempt will now be made to explain the action of iron which is present in the curing salts as an impurity, unknowingly added when the salted skin is weighted with earth. Malabar soil is essentially laterite and is naturally rich in iron oxides, but insoluble iron is not by itself capable of producing a stain. Soluble iron salts alone are also ineffective, so there must be an agency which fixes the soluble iron salts on the hide and transforms them so as to impede their removal by water. The part played by bacteria though not to be overstressed is by no means an unimportant one. Iron present in an insoluble form in the soil and accompanying the curing salt is solubilised by the activity of micro-organisms. Starkey and Halvorson (*Soil Sci.*, 1927, **24**, 381 ; *ibid.*, 1931,

1

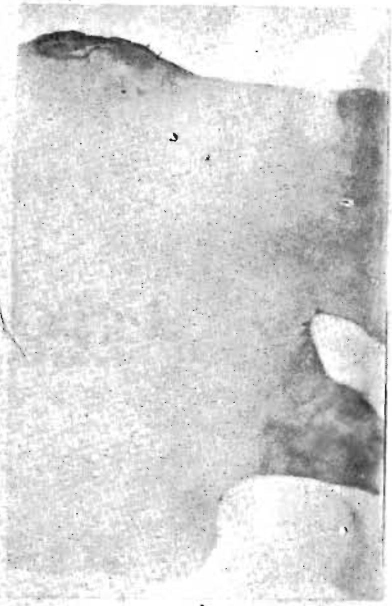


2

1 and 2. Pieces of limed and dehaired hide showing natural stains.

3

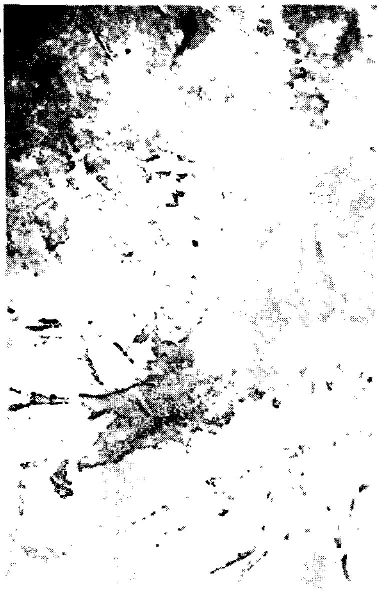
A piece of limed and dehaired skin showing artificial stains.



4

4. A piece of unstained Skin as control.

1



2

1 and 2. Pieces of limed and dehaired hide showing natural stains.

3

A piece of limed and dehaired skin showing artificial stains.

4

4. A piece of unstained Skin as control.

32, 141) in an extensive study on the transformations of iron in nature have found that insoluble iron can be brought into solution and reprecipitated in the form of ferric hydroxide by the activities of micro-organisms other than iron bacteria. They emphasise particularly a very clear distinction, first made by Winogradsky (*Centralbl. Bakt.*, 1922, II, **57**, 1), between iron bacteria and organisms which may be associated in the precipitation of iron. They have shown that under ærobic conditions micro-organisms may effect solution of iron by the acid produced, while under anærobic conditions in dextrose or peptone media, micro-organisms may dissolve and reduce iron present as ferric hydrate by decreasing the oxygen pressure and by the formation of acid, even at reactions approaching neutrality. Such conditions are likely to occur in the curing pits or godowns, where a considerable quantity of iron may pass into solution either from the soil or from the cement lining. According to the same authors the subsequent precipitation of iron under ærobic conditions may or may not be dependent upon microbial activity, while at the same time iron combined with organic radicals may become precipitated owing to action of organisms on the radicals; in which case an oxidation before precipitation might not be necessary.

In light of our own observations the process of stain-formation may be pictured as follows:—The micro-organisms find a rich medium in the hide for their growth, and due to their metabolic activities the oxygen tension in the medium is reduced, with simultaneous production of acids which tend to bring the insoluble iron into solution; this then probably combines with the hide substance. Now, accompanying the decomposition of protein matter there is an appreciable production of hydrogen sulphide which might combine with the soluble iron compound to form ferrous sulphide. Ferrous sulphide so formed, does not precipitate itself either in cures or in soaks, because the reaction is slightly acid, *i.e.*, less than P_H 6.6. In lime liquors, however, the reaction becomes sufficiently alkaline to precipitate the ferrous sulphide on the skin. The distribution of the stain on the grain surface is uneven, and may vary from pin points to large continuous patches. In many cases the stain gives an impression of bacterial colonies. The appearance of stains in such different patterns suggests that the solubilised iron becomes localised by the dense mass of hair or the close packing of the hides, which prevents its distribution, and it then combines with the hide substance as explained by Abt (*loc. cit.*). Whether the iron exists merely as ferrous sulphide in the stains, ferrous sulphide adsorbed on the hide substance or combined chemically with the latter, is at present difficult to say, but if iron exists as free ferrous sulphide in solution, it ought to be washed away while the hides are being soaked. Since it is not removed by washing, it is possible that it may have combined with the hide substance, or may have been adsorbed by it.

That stains are caused by ferrous sulphide has been proved. Pieces of sheep-skin were cured in pure brine for a month, when they were washed and limed; on de-hairing the surface was unstained. They were then immersed for three days in a weak solution of ferric chloride from which they were washed free, showing faint yellow patches resembling the colour of the ferric

chloride solution. On remaining several days in lime the patches retained their colour. One piece was kept as control and others were removed to fresh lime containing a very small quantity of sodium sulphide ; immediately the yellow patches became deep blue, much resembling the colour of the stains observed. The evidence thus points to the conclusion that stains may be caused by ferrous sulphide and not by ferric hydroxide.

Suggestions for prevention of stain-formation.

From the experiments described in a previous section it will be apparent that, using identical materials, stains developed only under conditions favouring the various transformations of iron. On the basis of our observations we suggest the adoption of the following procedure to lessen the incidence of stains :—

(1) Curing in pits should be discontinued, or practised with greater cleanliness so as to avoid laterite soil being associated with the hides or the curing salt.

(2) The godowns should be provided with adequate drainage and better ventilation to diminish the humidity.

(3) A better grade of common salt should be used for curing. The salt used in dry operations must be free from iron as well as magnesium salts which impart hygroscopic properties. The application of once used salt adulterated with soil should be discouraged.

These precautions if carefully observed, will, in our view, reduce the percentage of stained hides to a large extent. The question of de-staining has already been discussed (Patwardhan, *Chem. Ind.*, 1931, **50**, 722).

Summary.

The conditions of curing operations in Malabar leading to stain-formation have been described. The experimental evidence so far points to the following with regard to the origin and the nature of the stains :—(*a*) the stains are not due to direct bacterial action on the skin, (*b*) the presence of iron in the cures leads to stain-formation, and (*c*) stains are caused by precipitation of iron in the hide as ferrous sulphide.

A few suggestions for preventing stain-formation have been made.

The work is still in progress and the various problems arising out of the present investigation will form the subject of later communications.

The authors have great pleasure in thanking Prof. V. Subrahmanyan for his many useful suggestions and kindly interest in the work, and Mr. A. Guthrie, Special Officer on Salt Stains with the Madras Government, for supplying extensive information on the curing operations in Malabar and helping with various suggestions. They also thank the Director of Industries, Madras, through whose kindness one of them (M. S. S.) was enabled to take part in the investigation.

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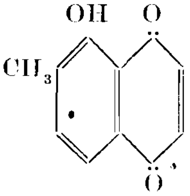
CHEMICAL EXAMINATION OF THE ROOT-BARK OF *PLUMBAGO ROSEA*, LINN.

By M. C. Tummin Katti and V. N. Patwardhan.

Chitramul (*Plumbago rosea* or *P. zeylanica*) is well known in Ayurvedic and Unani systems of medicine ; the history, description, uses and the early literature up to 1889 have been given in *Pharmacographia Indica*, Vol. II, p. 328. Plumbagin was isolated from *Plumbago rosea* by W. Benttink in 1888 as a yellow, crystalline compound melting at 72° and having the molecular formula, $C_{16}H_{13}O_6$. In 1928 (*Proc. Ind. Sci. Congress*, 1928, **15**, 163) one of us (M.C.T.K.) communicated a preliminary examination of *Plumbago zeylanica* root, wherein the isolation of purified plumbagin (m.p. 78°) giving a benzoyl derivative melting at 146° was reported. Madinaveitia and Gallego (*Anal. Fis. Quim.*, 1928, **26**, 263) obtained plumbagin (m.p. 76°) from *Plumbago europea* and have described some of its properties and several derivatives. They are of opinion that plumbagin, to which they assign the formula $C_{11}H_8O_3$, is probably methyljuglone, the exact position of the methyl group being uncertain ; it should therefore have one of the following three formulæ :—



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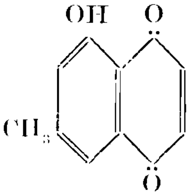

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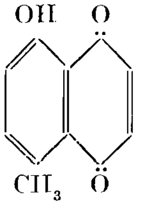

III

Roy and Dutt (*J. Ind. Chem. Soc.*, 1928, **5**, 419) isolated plumbagin (m.p. 77–78°) from *Plumbago rosea* and *Plumbago zeylanica*, describing its properties and derivatives. Originally they assigned the formula $C_{18}H_{15}O_5$ to plumbagin, but later (*Chem. Ind.*, 1929, **7**, 40) changed it to $C_{18}H_{16}O_5$, when an objection was raised by Klein (*ibid.*, 1928, **6**, 1035) that the original formula did not satisfy the law of even atom numbers. From experimental evidence they are of opinion that plumbagin may be a lactone in addition to containing hydroxy- and para-quinone groupings.

Since it was apparent from the foregoing brief review that a systematic investigation of the drug is needed and that the information regarding the chemical nature of plumbagin itself is conflicting, it was deemed desirable to subject the root of *Plumbago rosea* to further examination.

Experimental.

The material employed for this investigation consisted of the dark brown root-bark obtained from the local market.

Preliminary examination : Test for alkaloids.—The ground material (15 g.) was digested with Prollius's fluid for two hours, when the filtrate, examined

oleate and linoleate. The first fraction and the remaining three combined were separately hydrolysed and oxidised by cold alkaline permanganate solution. The hydroxy-acids thus obtained in each case were extracted in a Soxhlet apparatus successively with petroleum ether, ethyl ether and ethyl alcohol. Only a small amount of dihydroxystearic acid (m.p. 129°) could be obtained from the first fraction. From the last three fractions, an acid melting at 167–169° and having the molecular weight 347.2 was obtained in addition to dihydroxystearic acid. The molecular weight of this acid corresponds to that of a tetrahydroxystearic acid, but the melting point is slightly lower than that of sativic acid ; a low melting point of tetrahydroxystearic acid has been observed before by several investigators, and it is therefore possible that this product is a mixture of isomeric acids having the formula, $C_{18}H_{36}O_6$.

Ethyl Ether Extract.

The solvent from the extract was removed and the residue distilled with steam, yielding only plumbagin ; the residue was filtered and the filtrate extracted with chloroform. Attempts were made to dissolve the brown, water-insoluble residue in this chloroform solution, but with only partial success. The brown residue was amorphous, insoluble in water and only slightly soluble in ether or chloroform, but dissolved very readily in sodium hydroxide solution giving a dark red solution. Attempts to crystallise this material from alcohol and acetone failed.

The chloroform solution was extracted with ammonium carbonate and sodium hydroxide solutions ; the former extract on acidification gave a very small amount of semi-solid material while the latter yielded more of the brown material. The chloroform solution after extraction with alkaline solutions was distilled, and the residue saponified with alcoholic sodium hydroxide solution, the dry soap being extracted in a Soxhlet apparatus with dry ethyl ether, a white crystalline material separating from the extractive.

Sitosterol-glucoside, a phytosterolin, $C_{33}H_{56}O_6$.—Concentration of the ether extract gave a further quantity of the white substance which, after being crystallised several times from alcohol, yielded a colourless product melting at 259–260°. It gave the usual colour reactions of phytosterols and this feature, coupled with its high melting point, suggested a phytosterolin. It was therefore hydrolysed with amyl alcoholic hydrochloric acid in the manner described by Power and Salway (*J.C.S.*, 1913, **103**, 404) in connection with the hydrolysis of ipuranol. The phytosterol thus obtained melted at 135–136° and was identical with that isolated from the petroleum ether extract before described. The aqueous acid liquid yielded glucosazone.

The ethereal filtrate after the separation of sitosterol-glucoside was concentrated and the residue after some time deposited some crystalline material which, on several crystallisations from alcohol, melted at 77–78° with previous softening. Its insolubility in concentrated sulphuric acid even after warming on the water-bath suggested that it might be a saturated hydrocarbon, but the quantity was too small for analysis.

Chloroform, Ethyl Acetate and Acetone Extracts.

The residue after removing the solvent gave a small amount of plumbagin on steam distillation ; the water-insoluble residue consisted mostly of a

brown amorphous powder similar to that isolated from the ethyl ether extract. Ethyl acetate extracted only traces of plumbagin and the brown amorphous powder, while the acetone extract consisted almost entirely of the brown amorphous powder.

Ethyl Alcohol Extract.

The extract after removing the solvent was divided into water-soluble and water-insoluble portions, the latter consisting of brown material only. The former was precipitated with lead acetate solution and the precipitate de-leaded, when the filtrate from lead sulphide gave a green colour with ferric chloride solution indicating the presence of tannin. The filtrate from the lead precipitate was freed from excess of lead with hydrogen sulphide and concentrated under reduced pressure to a syrup, which reduced Fehling's solution abundantly and readily yielded an osazone melting at 204–205°, thus indicating glucose.

Properties of Plumbagin.

Further crystallisation did not raise the melting point of plumbagin, though a specimen isolated from *P. zeylanica* by one of us melted at 78°. The substance is freely soluble in ether, chloroform, benzene and alcohol, sparingly in cold water and moderately soluble in hot water ; it dissolves in alkaline solutions giving a cherry-red colour. The original compound can be recovered if the alkaline solution is acidified immediately, otherwise the colour gradually changes to red and finally brown, the solution on acidification then yielding a brown, tarry material.

Plumbagin is volatile with steam and this property affords the best method of purification. Our analyses favour the empirical formula of Madinaveitia and Gallego (Found: C, 70.0 and 70.1 : H, 4.1 and 4.3. $C_{11}H_8O_3$ requires C, 70.2 ; H, 4.3. $C_{18}H_{16}O_5$ requires C, 69.1 ; H, 5.1 per cent.). The molecular weight as determined by the freezing point depression method (in benzene) was found to be 182 (mean of 178, 188, 179 and 184), that calculated for $C_{11}H_8O_3$ being 188 and for $C_{18}H_{16}O_5$, 312.

Solutions of plumbagin give an intense red colour with ferric chloride solution indicating the presence of a phenolic group. Attempts to prepare the acetyl derivative were not successful, but the compound very readily yielded pale yellow needles of the benzoyl derivative when treated with benzoyl chloride in pyridine solution. Plumbagin does not contain a methoxy- or ethoxy-group ; it yields a pale yellow carbethoxy-derivative melting at 108–109°.

An alcoholic solution of plumbagin when treated with bromine water gave an orange-red bromo-derivative which after three crystallisations from alcohol melted at 172–173° to a brown liquid and contained 44.4 per cent. of bromine. This compound is also obtained quantitatively when a saturated aqueous solution of plumbagin is treated with bromine water, and is evidently different from the dibromo-derivative of Roy and Dutt (*loc. cit.*) which melted at 122° and contained 34.4 per cent. of bromine.

Physiological properties.—Of all the compounds isolated from the drug during our work, only plumbagin and the brown powder appeared to possess any unusual physiological properties. The brown powder was found to be inert for all practical purposes by organoleptic tests. Plumbagin on the other

hand seemed to possess marked *physiological activity.* It has a very irritating odour and acts freely on the mucous membrane.

For testing its physiological activity, preliminary experiments were conducted to ascertain *the minimum dose of plumbagin lethal* to various experimental animals. The M.L.D. for albino rats was determined by injecting subcutaneously a 2 per cent. solution of the substance in 95 per cent. alcohol. The results are given in the following table :—

Weight of rat (grams)	c.c. injected	Result
216	1.00	Survived.[1]
278	1.00	Survived.[1]
253	1.00	Death after 2 days.
113	0.50	Survived.
105	0.50	Survived.
126	0.65	Survived.
70	0.35	Survived.
208	0.50	Survived.
102	0.50	Death after 18 hours.
82	0.50	Death after 10 hours.
57	0.35	Death after 15 minutes.[2]
68	0.40	Death after 40 hours.
82	0.50	Death after 20 hours.

[1] Urine was intensely yellow for a day and contained plumbagin.

[2] Intestinal puncture.

Three rats weighing roughly 100 grams each were injected with 0.5 c.c. of 95 per cent. alcohol for control, and all survived. The M.L.D. for rat seems to be between 110 and 120 milligrams of plumbagin per kilogram of body-weight. Similarly the M.L.D. for guinea pigs and frogs was found to be about 20 and 35 milligrams respectively per kilogram of body-weight. The number of animals used in these cases being small, the results could be taken as only indicative.

The post mortem examination of the animals in each case showed that *plumbagin acted chiefly* on the respiratory system. We hope to make a systematic pharmacological examination of this compound at a later date.

Summary.

The alcoholic extract of the root-bark of *Plumbago rosea* has been found to contain sitosterol, a fatty alcohol (probably arachidyl alcohol), oleic, linoleic and probably lignoceric acids, a phytosterolin (sitosterol-glucoside), a saturated hydrocarbon, glucose and a large amount of a brown, amorphous, inert material. Our analysis of plumbagin did not confirm the formula assigned to it by Roy and Dutt, but indicated that of Madinaveitia and Gallego as more probably being correct. Plumbagin seems to be the only compound in the root-bark responsible for the physiological activity of *Plumbago rosea.*

We wish to express our grateful thanks to Dr. C. V. Natarajan, Chemical Examiner to the Government of Mysore and Superintendent, Public Health Institute, Bangalore, for according us the facilities of the Public Health Institute Laboratories, and actively helping us in the methods and technique of animal experimentation. We also desire to record our appreciation of the keen interest shown by Professor Subrahmanyan in the present investigation.

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DILATOMETRIC STUDIES IN ENZYME ACTION. PART II. CONTRACTION CONSTANTS OF ENZYME-SUBSTRATE REACTIONS.

By M. Sreenivasaya and H. B. Sreerangachar.

In a previous communication (*Biochem. J.*, 1929, **23**, 975) it was shown that hydrolysis of substrates by their respective enzymes is generally accompanied by measurable changes of volume. At lower concentrations of the substrate, the total volume change brought about by an enzyme-substrate reaction is proportional to the weight of the substrate present in the reaction mixture and is not affected by temperature or concentration of enzyme, hydrogen and other ions, all of which are known to influence the kinetics of the reaction. In the case of crystalloidal substrates whose molecular weight is known, the total contraction is expressed in cubic centimetres per gram-molecule of the substrate and this is a constant for a given enzyme-substrate system. In the case of colloidal substrates, starch, proteins, etc., the contraction constant is expressed in cubic centimetres per 100 grams of the substrate.

The Dilatometer.

The dilatometer employed successfully for time-course studies of enzyme action in previous work was found unsuitable for the present investigation, where we are concerned with the total volume change and not with the rate of reaction at a given moment. In previous studies the enzyme and substrate were mixed outside the dilatometer in a separate vessel at the temperature of the thermostat, after which the apparatus (Fig. I) was filled with the reacting mixture by suction at the capillary end. The whole operation takes 2 to 3 minutes, and, under the most favourable conditions of manipulation, the first reading on the dilatometer can be taken only after 3 minutes. This procedure, therefore, does not bring into consideration the substantial volume change effected (1) on mixing and (2) by reaction during the first three minutes. In some instances the fall is so rapid that nearly 30-35 per cent. of the volume change takes place during this period as shown in Table I.

TABLE I.

Reaction	Volume change occurring after 30 secs.	Volume change occurring after 3 mins.	Total	Percentage of reaction during 3 mins.
Urease-urea ..	1.5	14.3	39.0	36.6
Invertase-sucrose ..	0.0	3.4	12.1	28.1

When the investigation was in progress Rona (*Biochem. Zeit.*, 1931, **235**, 214), employing the same dilatometer and procedure as above, obtained values for invertase-sucrose and maltase-maltose systems which unexpectedly coincide with ours derived from the new instrument.

To determine the total volume change, therefore, it is necessary that the substrate and the enzyme should be mixed in the dilatometer itself, after the first reading of the meniscus in the capillary is taken. These conditions have been fulfilled by designing a new type of instrument, which has been made for us by Messrs. Greiner and Friedrichs.

The New Type.

The new type of dilatometer (Fig. II) consists of an inverted Y-shaped vessel, one of the arms being blown into a pear-shaped bulb S, of about 75 c.c. capacity to receive the substrate. The adjacent arm is blown into a smaller bulb E, to contain 10–15 c.c. of the enzyme extract, while the third arm, pointing upwards, ends in a mouth provided with a carefully ground-in glass stopper. A 450 mm. length of capillary of uniform bore (0.4 mm.) is attached at the upper end of the pear-shaped bulb.

EXPERIMENTAL.

The dilatometer, more especially its capillary, is carefully cleaned successively with alcoholic potash, chromic acid, water, alcohol and finally with ether. The capillary is calibrated in the usual way and the observed mean cross-section is used in calculating the volume change accompanying a reaction. The bulbs of the dilatometer S and E are filled with the substrate and the enzyme extract respectively, employing the filler (Fig. II) provided with the instrument; the filler being rinsed each time with 2 c.c. of toluene to wash down substrate or the enzyme extract as the case may be. During the filling great care should be taken to avoid mixing the two components.

The substrate consists of a known weight of the purified substance, usually a twentieth of the gram-molecule dissolved in a suitable buffer at the optimum P_H and diluted to a litre with the same buffer solution. The substrate solution, 40–50 c.c., depending upon the amount of substance dissolved, and 5-10 c.c. of the enzyme extract depending upon its activity, are employed for each experiment. The dilatometer is afterwards stoppered with a rubber cork provided with a T-tube, to one end of which is attached a separating funnel (Fig. III). The other end of the tube is connected to a suction pump by means of pressure-tubing controlled by a screw pinchcock. The capillary end is closed by a short piece of rubber tubing closed at one end by a glass rod, and suction is applied with a filter pump to remove the gases dissolved in the liquids. During the operation, which usually takes 30 minutes, the toluene layer covers the surfaces of the substrate and enzyme extract, facilitating the smooth escape of gas bubbles and preventing foaming or spraying, more particularly, of the colloidal extract. At first great care should be exercised in controlling the suction by means of the screw pinchcock, as otherwise too rapid a removal of the gases may cause spraying and mixing of liquids. When bubbles of gas cease rising in the two liquids, suction is cut off by means of the pinchcock, and high boiling petrol (110—120°), distilled successively over potash and mercury, slowly admitted to the dilatometer (Fig. III) until the remaining air space is filled. A few glass beads are finally added and the apparatus immersed in the thermostat maintained at $30° \pm 0.01°$. After half an hour, the dilatometer is closed with the stopper smeared with a special grease mainly composed of the resin acids of shellac which, by virtue of its insolubility in petrol, prevents the solvent from creeping through the ground glass stopper fastened in position

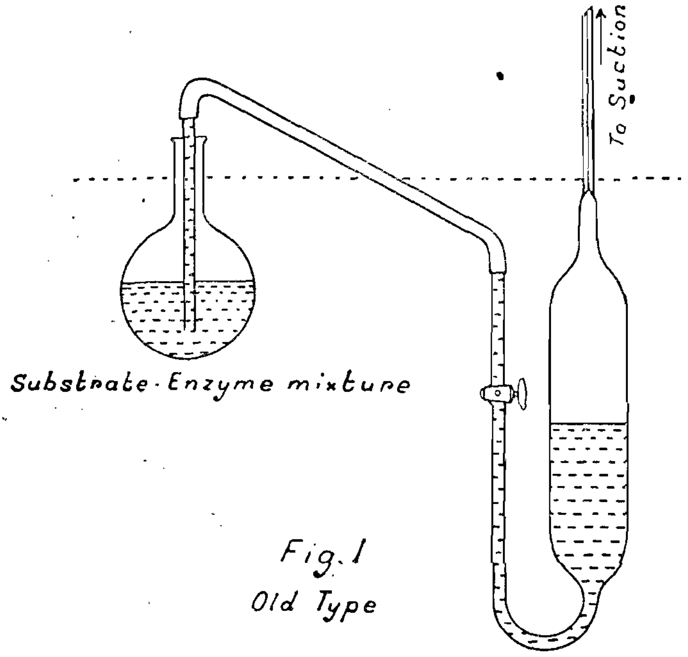

Fig. I
Old Type

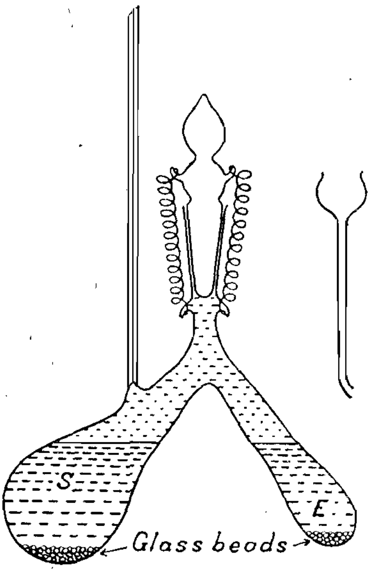

Fig. II

New Type

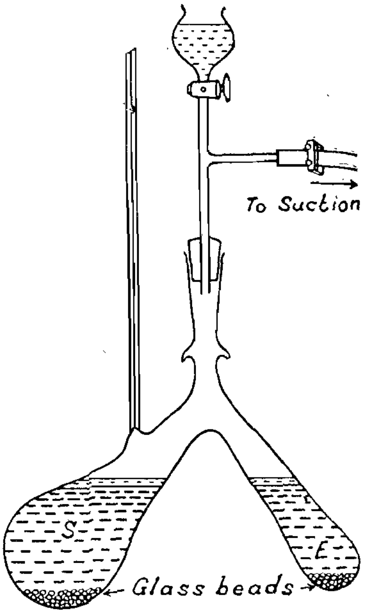

Fig. III

by two springs (Fig. II). A control experiment showed the efficiency of the grease, the meniscus in the capillary varying within the same limits for more than 48 hours. All the dilatometers employed in the investigation were subjected to this test.

The meniscus in the capillary, which usually reaches the top, is adjusted to a convenient lower level by warming the larger bulb by hand, when a portion of the petrol flows out at the capillary end. On regaining the temperature of the thermostat, the meniscus falls to a lower level. The extent of adjustment is to be determined by the nature of the reacting components ; while in some cases there is an almost instantaneous fall in the dilatometric column, a definite and appreciable rise is observed in a few others.

After adjusting the meniscus, the limits of variation of the dilatometric column due to the temperature variations of the thermostat are determined. The combined volumes of the substrate, enzyme and petrol, more particularly on account of petrol possessing a high coefficient of cubical expansion, amounts to nearly 100 c.c. which renders the instrument highly sensitive even to small changes of temperature falling within $\pm 0.01°$; but so long as the variations lie within definite limits, as has been found to be the case, they do not interfere with our experiments. The range of temperature variation, as indicated by the maxima and minima of the meniscus, is determined and agreement between the three sets of consecutive results is an assurance that the entire contents of the dilatometer have attained the temperature of the thermostat. Subsequent readings after mixing are taken either at the maximum or the minimum temperature as indicated by the control dilatometer which is simultaneously maintained in the thermostat, the same quantities of the boiled enzyme, substrate and petrol being employed. Table II shows the readings for successive maxima and minima of the experimental and control dilatometers in a typical experiment.

TABLE II.

		Experimental	Control	Water Control
		cms.	cms.	cms.
Before mixing ..	max.	5.1	8.8	15.6
,, ..	min.	4.3	8.2	15.5
,, ..	max.	5.2	8.9	15.6
,, ..	min.	4.3	8.2	15.5
,, ..	max.	5.1	8.9	15.6
,, ..	min.	4.3	8.2	15.5
,, ..	max.	5.2	9.0	15.6
,, ..	min.	4.3	8.2	15.5
24 hrs. after completion.	max.	15.5	9.0	15.6
,,	min.	14.7	8.2	15.5
,,	max.	15.5	9.0	15.6
,,	min.	14.7	8.2	15.5
,,	max.	15.5	9.0	15.6
,,	min.	14.7	8.2	15.5

The reaction components in the dilatometer are then mixed at a definite temperature, the maximum or the minimum shown by the control dilatometer

holding the capillary by means of a cloth and tilting the apparatus underneath the water of the thermostat. The glass beads and the petrol help the mixing which can be effected in about 30 seconds, during which period the meniscus can be easily read. Subsequent readings at known intervals can be taken. Thus it is possible to study not only the kinetics of enzyme action almost from the moment of mixing, but also the total change occurring at the end of reaction. This instrument has the further advantage of revealing to some extent the abnormal and interesting changes occurring in the initial stages of the reaction on mixing. Table III indicates some of the notable changes occurring in a few of the enzyme-substrate systems immediately on mixing.

TABLE III.

Reaction	Change observed 30 secs. after mixing	
	Experimental	Control
1. Arginase-arginine	+10 mm.	+8 mm.
2. Diastase-starch	++	+
3. Emulsin-amygdalin	0.0 mm.	0.0 mm.
4. Emulsion-salicin	++++	++
5. Invertase-sucrose	0.0 mm.	0.0 mm.
6. Urease-urea	—15 mm.	—5 mm.

+ or — respectively indicates rise or fall in the dilatometric column.

It is difficult, at this stage, to offer any explanation of these initial changes, which are being subjected to a detailed investigation.

The course of hydrolysis can then be followed by taking readings of the dilatometric column at known intervals. The reaction reaches equilibrium in the course of 6–8 hours, after which a final reading of the dilatometric column is recorded and the difference between the first and the final readings gives the total fall in the dilatometric column. From this the total reduction of volume V in cubic centimetres may be calculated. If the weight of the substrate W present in the reaction is known, the contraction constant K is given by the formula,

$$K=\frac{V}{W}M$$

where M represents the molecular weight of the substrate. In the case of colloidal substrates M is replaced by 100.

Sources of Error.—All possible sources of error in these dilatometric estimations have been carefully examined. Errors due to progressive changes of surface tension in the reacting mixture are not taken into consideration in these experiments since the mixture does not come into contact with the capillary containing the high boiling petrol. Evaporation from the capillary meniscus and drained surfaces was found to be negligible. The error caused by fluctuations of atmospheric temperature in the exposed capillary column has been found to have the order of ±0.15 mm. per degree change for an exposed column of 20 cms., which is about the average height attained in our experiments. Sources of error seriously affecting the constant are: (1) purity

of the substrate and (2) percentage of hydrolysis brought about by the enzyme under the conditions of the experiment. The purity of the substrate is ensured by repeated recrystallization and the percentage of purity determined by an independent analysis. The degree of hydrolysis is found by a series of experiments employing a method entirely independent of the dilatometer ; in the case of urease-urea, for example, the aeration method of estimating urea is used. At each concentration of substrate employed, the percentage of hydrolysis was determined, and the correction for complete hydrolysis calculated ; this correction is useful in estimating the substrates of unknown physiological fluids.

The present communication describes the investigation of two closely allied enzyme-substrate systems : (1) urease-urea and (2) arginase-arginine. For a study of system (1) the following materials were employed :—(*a*) The enzyme urease, from Arlco Jack bean preparation in tablet form, and (*b*) Urea (B.D.H.) which, twice recrystallized from alcohol was found to be 99.8 per cent. pure by two independent methods, (1) total nitrogen by Kjeldahl and (2) aeration method of Van Slyke and Cullen (*J. Biol. Chem.*, 1914, **19**, 211).

The reaction mixture consisted of 40 c.c. of a solution prepared by dissolving a known weight of urea in Sorensen's phosphate buffer (P_H 7.0) and 5 c.c. of a 1 per cent. solution of the urease preparation. The experimental procedure before described was closely followed. Table IV gives the results for the urease-urea system while Table V records those of aeration studies carried out under similar conditions to determine the percentage of hydrolysis effected by the system.

TABLE IV.

Concentration of Substrate	Initial reading		Final reading		Total fall in the dilatometric column	Contraction constant (uncorrected)
	Exptl.	Control	Exptl.	Control		
	mm.	mm.	mm.	mm.	mm.	
1. *M*/20	10.0	9.0	400.0	9.0	390.0	23.96
2. *M*/20	9.0	12.5	398.0	12.5	389.0	23.90
3. *M*/20	12.0	25.0	401.5	25.0	389.5	23.93
4. *M*/30*	42.0	48.0	365.5	48.0	323.5	23.92
					Average ..	23.93

*50 c.c.

Corrected for purity and percentage of hydrolysis .. 24.13

TABLE V.

	Urea taken in mgms.	Urea found in mgms.	Percentage of hydrolysis
1	30	29.82	99.4
2	30	29.83	99.4
3	15	14.88	99.2
		Average ..	99.3

The following materials were employed for a study of the arginase-arginine reaction. The enzyme was obtained from sheep's liver as the glycerol extract, by the method of Hunter and Daulphinee (*J. Biol. Chem.*, 1929-30, **85**, 627). The substrate was derived from two sources : (1) B. D. H. "arginine", which experiment showed to consist of *d*- and *l*- arginine in equal amounts (see Table VI), and (2) Dr. Theodor Schuchardt "*d*- arginine". The purity of the two samples of arginine has been ascertained by (1) a determination of total nitrogen by Kjeldahl and (2) the aeration method as described by Hunter and Daulphinee.

TABLE VI.

Percentage of Arginine.

Source	Kjeldahl	Aeration
B.D.H.	101	49.64
Schuchardt ..	100	99.98

The reaction mixture consisted of 50 c.c. of $M/20$ arginine solution prepared by dissolving the requisite amount of arginine in phosphate buffer (P_H 8.04) and 5 c.c. of the enzyme extract. The experimental procedure adopted was the same as described before and the results are given in Table VII.

TABLE VII.

Sample		Initial reading		Final reading		Total depression	Constant (un-corrected)
		Experimental	Control	Experimental	Control		
		mm.	mm.	mm.	mm.	mm.	
B.D.H.	1 ..	31.0	22.0	82.0	22.0	51.0	2.5
	2 ..	45.0	76.0	122.0	76.0	52.0	2.55
						Average ..	2.53
Schuchardt	1 ..	88.5	95.0	191.5	95.0	103	5.06
	2 ..	84.0	71.0	185.0	71.0	101	4.98
	3 ..	72.0	40.0	174.0	40.0	102	5.01
						Average ..	5.02
		Corrected for purity and percentage of hydrolysis				..	5.02

The contraction constants of other enzyme-substrate systems are being determined.

SUMMARY.

1. A new type of dilatometer has been described where the enzyme and the substrate could be mixed in the dilatometer itself, thereby facilitating an investigation of the changes from the beginning of the reaction. The kinetics of the reaction could simultaneously be followed.

2. At lower concentrations of substrate, the total contraction brought about by the system is found to be proportional to the weight of the substrate present in the reaction mixture. The contraction in cubic centimetres due to the enzymic hydrolysis of a gram-molecule of the substrate can be calculated, and represents the contraction constant of that particular enzyme-substrate system.

3. The experimental procedure has been described in full detail ; various sources of error affecting the determination have been carefully examined, and the corrections applied wherever found necessary.

4. The contraction constants of two closely allied enzyme-substrate systems, urease-urea and arginase-arginine, have been found to be 24.13 and 5.02 respectively and the usefulness of these constants in a determination of urea and arginine in unknown physiological fluids and protein hydrolysates is indicated.

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ARYL DI- & POLY-STIBINIC ACIDS, DISTIBINOUS OXIDES AND DISTIBINO-COMPOUNDS.*

By Sohrab M. Mistry and Praphulla Chandra Guha.

The importance of organic antimonials as trypanocides was discovered when Plimmer and Thomsen (*Proc. Roy. Soc.*, 1908, B **80**, 1) found that trypanosomes rapidly disappeared from the blood of infected animals on injection with antimony compounds. The importance of the stibinic acids as trypanocides has stimulated research from the standpoint of chemotherapy. From the little work that has been done, very few conclusions could be drawn as regards the relationship between chemical constitution and therapeutic properties of organo-antimony compounds. The stibinic acid grouping $-SbO(OH)_2$ has the characteristic trypanocide property, but it is believed that this is due to the formation *in vivo* of trivalent $-Sb{=}O$ as the real trypanocide (*cf.* Brahmachari, *Ind. Sci. Congress, Med. Sec.*, 1931). It is an established fact, however, that the substituents in the nucleus of an aromatic stibinic acid also exert a marked influence. Thus the introduction of an amino-group in the aryl nucleus of a stibinic acid diminishes toxicity to a considerable extent, and acetylation of the amino-group increases the diminution. The acetylated product, however, being more unstable becomes toxic on keeping and hence useless as a therapeutic agent (Brahmachari, *Ind. J. Med. Res.*, 1922, **10**, 521 ; also *loc. cit.*). In the arsenic series the acetylation of an amino-group renders the compound more active. Stibinic acids substituted both by chloro- and amino-groups are also active anti-kala-azar remedies. Again, stibinic acids derived from aminobenzylbenzoates and benzylaminobenzoate have been found to be without any marked curative action on kala-azar (Niyogy, *J. Ind. Chem. Soc.*, 1930, **7**, 577).

Although the discovery of the most important synthesis of stibinic acids by the diazo-reaction (first applied by Bart, 1910, to the synthesis of arsonic acids) is attributed to the Chemische Fabrik von Heyden (1912, *D. R. P.*, 254,421), P. May was the first to publish a paper (*J. C. S.*, 1912, **101**, 1032) describing a few double salts of antimony trichloride and aryl diazochlorides. The alkali treatment of these double salts would have given him the corresponding stibinic acids ; but this was first mentioned in a German patent (*D. R. P.*, 261,825). A thorough discussion of the reactions involving the formation of a stibinic acid by the diazo-reaction is due to H. Schmidt (*Annalen*, 1920, **421**, 174 ; **429**, 123 ; *Ber.*, **55B**, 697 ; *ibid.*, **57B**, 1142 ; *ibid.*, **59**, 555).

Urea stibamine discovered by Brahmachari (*Ind. J. Med. Res.*, 1922, **10**, 508 ; 1924, **12**, 423) to which the structure $NH_2.CO.NH.C_6H_4.SbO(OH)(ONH_4)$-*p* was originally attributed, has had phenomenal success in the cure of kala-azar. It has now been found that it is not an individual, but most probably a mixture (*J. Soc. Chem. Ind.*, 1930, **49**, 800). Niyogy on the other hand (*J. Ind. Chem. Soc.*, 1928, **5**, 757), however, claims to have obtained a

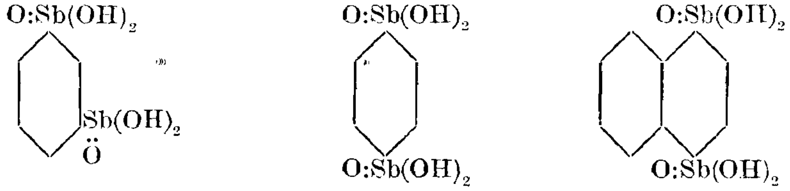
* Preliminary reports on this work have been published in the *Proceedings of the Indian Science Congress* (Chemistry Section, 1930, 176 ; 1931, 162), and have been communicated before the preceding 15th October of each year.

product by treating a hydrochloric acid solution of *p*-aminophenylstibinic acid with potassium cyanate which he considers to be identical with urea stibamine.

The present investigation was undertaken to prepare a series of compounds the pharmacological examination of which was likely to throw some further light on the chemotherapy of organic antimonials. If the stibinic acid grouping had any influence on the trypanocidal property it was thought worth while to investigate various types of aromatic compounds having more than one stibinic acid group in the molecule. The compounds prepared can be divided into five categories :—(*a*) where antimony is attached to one and the same aryl nucleus ; (*b*) where antimony is attached to different aryl nuclei directly connected ; (*c*) the same as (*b*) but with the aryl nuclei connected through one or more carbon atoms ; (*d*) two antimony-containing aryl groups connected through carbon and nitrogen atoms, and (*e*) antimony-containing aryl groups linked through nitrogen, phosphorus, arsenic or antimony.

Although mixed arsono-stibinic acids had been prepared by several workers in this field (*D. R. P.*, 296,940 ; Schmidt, *Ber.*, 1924, **57B**, 1147), no distibinic acid appeared to have been known when this work was undertaken. Dunning and Reid (*J. Amer. Chem. Soc.*, 1926, **48**, 2959) claimed to have obtained diphenyldistibinic acid from benzidine by tetrazotisation and subsequent stibination, but they did not give details of their method, while the percentage of antimony in the compound was 4 per cent. above the theoretical value. During the progress of this work, however, Riddel and Basterfield's paper (*Proc. Roy. Soc. Canada*, 1930, **23**, I (III), 45) came to our notice where they reported failure to obtain a distibinic acid from benzidine and described the preparation of distibinic acids from *pp'*-diaminodiphenylmethane and *p*-phenylenediamine.

m-Phenylenediamine, *p*-phenylenediamine and 1 : 4-naphthylenediamine have now been found to give the corresponding distibinic acids (I-III) :

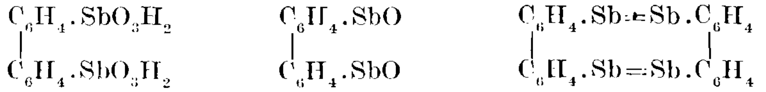


(I) (II) (III)

On repeating Dunning and Reid's work, it was found that the double compound of tetrazotised benzidine and antimony trichloride (*cf.* May, *J. C. S.*, 1912, **101**, 1037) on treatment with alkali gave an orange-brown, amorphous mass, the insolubility in alkali proving the absence of a stibinic acid group. Moreover, the compound was soluble in glacial acetic acid from which it could be precipitated by water as a light yellow powder ; it did not dissolve in concentrated hydrochloric acid but became deep violet, the colour disappearing on dilution with water, showing that the insoluble chloride is hydrolysed at lower concentration of the acid. Further, it was found that while alkaline hydrogen peroxide had no effect on this compound it is decomposed by more drastic oxidising agents. The above properties and the results of analysis support the distibinous oxide structure, $C_{12}H_8(SbO)_2$. Sodium thiosulphate reduces the

compound to a red powder which is completely decomposed without colouration by concentrated hydrochloric acid.

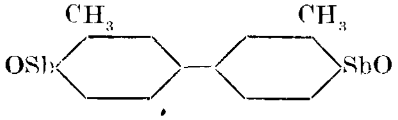
This is the first instance in which a trivalent antimonial has been obtained in a reaction which usually gives stibinic acids. Although the mechanism by which the distibinous oxide is formed is not quite clear, we suggest that most probably the distibinic acid is formed first by analogy with the Bart-Schmidt reaction and becomes easily reduced to the stable distibinous oxide, thus :—



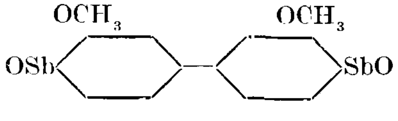
(IV) (IV *a*)

There being so much controversy on the structure of diphenyl and its derivatives, nothing can be said with certainty as to whether the space relation between the two stibinic acid groups in the *para*-positions of diphenyl is responsible for this anomalous behaviour. For comparison the diarsenic acid from benzidine was prepared and a good yield of the acid obtained.

Compounds (V and VI) similar to that obtained from benzidine were obtained from *o*-tolidine and dianisidine :—

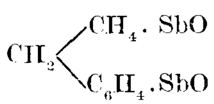


(V)

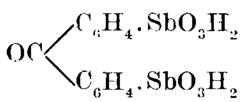


(VI)

Riddel and Basterfield (*loc. cit.*) claim to have isolated a distibinic acid from *pp*-diaminodiphenylmethane though they failed to purify their product ; our independent experiments before the paper came to our notice disagree with those of the above authors since we could isolate only a distibinous oxide (VII) and no stibinic acid. Our repeated experiments have only confirmed the above disagreement, but *pp*-diaminobenzophenone does give the corresponding distibinic acid (VIII).



(VII)



(VIII)

In the case of *pp*-diaminodiphenylmethane the distibinic acid is most probably formed, but reduction leads to the stable stibinous oxide form. In this connection it is interesting to note that the amino-groups of *pp*-diaminodiphenylmethane react easily with camphorquinone but those of *pp*-diaminobenzophenone do not (unpublished work). The influence of the methylene group in rendering the substituent stibinic acid grouping liable to reduction, in contradistinction to the stabilising effect produced by the carbonyl group, has been observed

in the antimony compounds obtained from *pp*-diaminodiphenylurea, *pp*-diaminodiphenylmethylenediamine, *pp*-diaminodiphenyloxamide and *pp*-diaminodiphenylethylenediamine (XIII, XIV, XVII and XV).

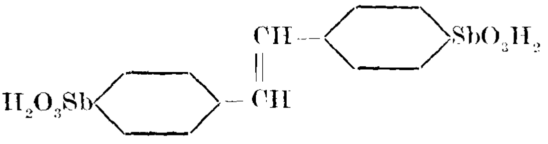
On being subjected to the Bart-Schmidt method of stibination, rosaniline yielded the expected tristibinic acid (IX) but leucaniline gave only a tristibinous oxide (X) to the complete exclusion of the expected stibinic acid.

$$HO.C\begin{cases} C_6H_4.SbO_3H_2 \\ C_6H_4.SbO_3H_2 \\ C_6H_3\,(CH_3).SbO_3H_2 \end{cases} \qquad HC\begin{cases} C_6H_4.SbO \\ C_6H_4.SbO \\ C_6H_3\,(CH_3).SbO \end{cases}$$

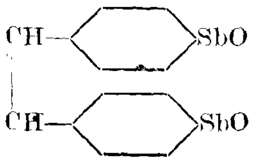
(IX) (X)

Reduction of the carbinol is evidently accompanied by a great change in property similar to that observed in the formation of compounds VII and VIII from diaminodiphenylmethane and diaminobenzophenone.

A distibinic acid (XI) and a distibinous oxide (XII) have been isolated from the product of stibinating *pp*-diaminostilbene. Formation of these different products may be explained by assuming that the *cis*-form of the diamine gives the distibinous oxide and the *trans*-form the distibinic acid. In the *cis*-form the two *para*-positions of the phenyl nuclei being so close together cannot retain two heavy acidic groups, whereas the *para*-positions of the *trans*-form being further apart can easily accommodate the stibinic acid groups :—



(XI)



(XII)

Compounds were next prepared in which the two stibinic acid groups were attached to two different aromatic nuclei separated from each other by a link of carbon and nitrogen atoms in varying number. The first compound of this series was prepared from *sym*-*pp*-diaminodiphenylurea, selected because the expected distibinic acid would be Brahmachari's supposed *p*-carbaminophenylstibinic acid with one more phenylstibinic acid grouping attached to the carbamido-group, and should possess trypanocidal properties in a much more pronounced degree. The double compound obtained by tetrazotisation and coupling with antimony trichloride on analysis agreed with the formula,

$$OC\begin{cases} NH.C_6H_4.N_2Cl,SbCl_3 \\ NH.C_6H_4.N_2Cl,SbCl_3 \end{cases}$$

and on treatment with alkali gave a sparingly soluble brown powder which became deep violet on treatment with concentrated hydrochloric acid, regaining its original colour on dilution. Analysis proved the presence of only one

atom of antimony in the molecule, however, and the formula corresponded to that of a monostibinic acid. To explain this peculiar reaction, *p*-monoaminodiphenylurea was stibinated by the usual method and a compound agreeing in all its properties with the stibinic acid prepared from *sym-pp*-diaminodiphenylurea was obtained. As is well known, it is extremely difficult to purify stibinic acids, and it is impracticable to prove the identity of stibinic acids obtained from two different sources; conclusions must be drawn from solubility and other physical properties in conjunction with results of analysis.

The mechanism of the reaction can be explained as follows: During alkali treatment of the double salt one of the $N_2Cl,SbCl_3$ groups is transformed as usual into a stibinic acid group while the other is replaced by an atom of hydrogen:

$$OC\left\langle\begin{matrix}NH.C_6H_4.N_2Cl,SbCl_3\\NH.C_6H_4.N_2Cl,SbCl_3\end{matrix}\right. \qquad OC\left\langle\begin{matrix}NH.C_6H_4.SbO(OH)_2\\NH.C_6H_4.N_2Cl,SbCl_2\end{matrix}\right.$$

$$OC\left\langle\begin{matrix}NH.C_6H_4.SbO(OH)_2\\NH.C_6H_5\end{matrix}\right.$$

The replacement of a diazo-complex by hydrogen is of common occurrence in such reactions and is not at all unexpected (*cf.* Christiansen, "Organic Derivatives of Antimony", p. 57).

A primary stibinic acid of this type would be readily soluble in alkali, but as has been mentioned above the product is sparingly soluble in sodium hydroxide solution. This fact could be better explained on the ground that a secondary stibinic acid is formed by the elimination of a molecule of water from the hydroxyl group of the phenyl-carbamino-4-phenylstibinic acid and the *para*-hydrogen atom of the other phenyl group, thus:—

$$OC\left\langle\begin{matrix}NH.C_6H_4.SbO(OH)_2\\NH.C_6H_5\end{matrix}\right. \qquad OC\left\langle\begin{matrix}NH.C_6H_4\\NH.C_6H_4\end{matrix}\right\rangle SbO.OH$$

(XIII)

Macallum (*J. Soc. Chem. Ind.*, 1923, 468 T) describes an analogous condensation and claims to have obtained a secondary stibinic acid as the main product of action between antimony trioxide and *o*-chlorobenzenediazonium chloride.

pp-Diaminodiphenylmethylene- and ethylenediamines gave distibinous oxides (XIV) and (XV) instead of the stibinic acids obtained from *pp*-aminodiphenylurea and oxamide

$$CH_2\left\langle\begin{matrix}NH.C_6H_4.SbO\\NH.C_6H_4.SbO\end{matrix}\right. \qquad \begin{matrix}CH_2.NH.C_6H_4.SbO\\|\\CH_2.NH.C_6H_4.SbO\end{matrix}$$

(XIV) (XV)

The formation of these two stibinous oxides is evidently due to the methylene and ethylene groups, the directing influence of which in the formation of

stibinous oxides in preference to stibinic acids having already been shown (*cf.* VII-X).

Bis-acetylaminophenylazomethine was obtained by treating two molecules of acetyl-*p*-phenylenediamine with one molecule of glyoxal, and on hydrolysis gave the corresponding diamine from which on stibination the distibinous oxide (XVI) was obtained :

$$\begin{array}{l} CH{=}N.C_6H_4.SbO \\ | \\ CH{=}N.C_6H_4.SbO \end{array}$$

(XVI)

pp-Diaminodiphenyloxamide, -malonamide, -succinamide and -carbohydrazide yielded the distibinic acids (XVII-XX) :

$$\begin{array}{l} CO.NH.C_6H_4.SbO_3H_2 \\ | \\ CO.NH.C_6H_4.SbO_3H_2 \end{array}$$

(XVII)

$$CH_2\left\langle \begin{array}{l} CO.NH.C_6H_4.SbO_3H_2 \\ CO.NH.C_6H_4.SbO_3H_2 \end{array} \right.$$

(XVIII)

$$\begin{array}{l} CH_2.CO.NH.C_6H_4.SbO_3H_2 \\ | \\ CH_2.CO.NH.C_6H_4.SbO_3H_2 \end{array}$$

(XIX)

$$OC\left\langle \begin{array}{l} NH.NH.C_6H_4.SbO_3H_2 \\ NH.NH.C_6H_4.SbO_3H_2 \end{array} \right.$$

(XX)

pp-Diaminodiphenylhydrazodicarbonamide, prepared for the first time, gave a distibinic acid on stibination in the usual manner :—

$$\begin{array}{l} NH.CO.NH.C_6H_4.SbO_3H_2 \\ | \\ NH.CO.NH.C_6H_4.SbO_3H_2 \end{array}$$

(XXI)

It will be seen that this is really a *bis*-compound of Brahmachari's urea stibamine, and as such is very likely to be a valuable trypanocide.

pp-Diaminohydrazobenzene on stibination gave a distibinous oxide :—

$$\begin{array}{l} NH.C_6H_4.SbO \\ | \\ NH.C_6H_4.SbO \end{array}$$

(XXII)

pp-Diaminobenzanilide has been prepared for the first time by the interaction of *p*-nitrobenzoyl chloride and acetyl-*p*-phenylenediamine, followed by reduction and hydrolysis. It gave on stibination the distibinous oxide (XXIII) :—

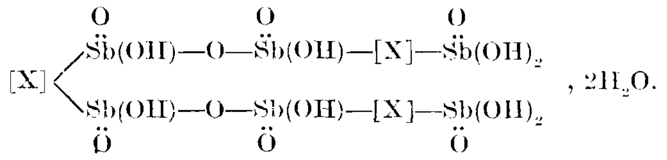
$$\begin{array}{c} NH.C_6H_4.NHAc \\ | \\ CO\ .C_6H_4.NO_2 \end{array} \rightarrow \begin{array}{c} NH.C_6H_4.NH_2 \\ | \\ CO\ .C_6H_4.NH_2 \\ \text{(XXIII)} \end{array} \rightarrow \begin{array}{c} NH.C_6H_4.SbO \\ | \\ CO\ .C_6H_4.SbO \end{array}$$

To study the effect of phosphorus, arsenic and antimony in *mmm*-triaminotriphenylphosphine, -arsine and -stibine respectively on stibination they have been subjected to the Bart-Schmidt method of stibination with the result that all of them have been found to yield the corresponding tristibinic acids (XXIV–XXVI). The combination of the three stibinic acid groups with other atoms of phosphorus, arsenic and antimony is likely to impart interesting pharmacological properties to these compounds. The triphenylphosphine, -arsine, and -stibine were prepared by a slightly modified method of Pfeiffer and his co-workers (*Ber.*, 1904, **37**, 4621). and the yields were considerably improved.

$$P\,(C_6H_4.SbO_3H_2)_3 \qquad As\,(C_6H_4.SbO_3H_2)_3 \qquad Sb\,(C_6H_4.SbO_3H_2)_3$$

(XXIV) (XXV) (XXVI)

In all cases the simple formula for the distibinic acids is given for the sake of saving space. According to Schmidt's complex formula for the stibinic acids they should be represented by a general formula of the type :



[X] representing the bivalent organic part of the molecule.

Experimental.

Of all the known methods for the preparation of stibinic acids by the diazo-reaction, the method of the *D.R.P.* 261,825 has been found to be the best. The amine is fully diazotised, a solution of antimony trichloride is added, the crystalline double salt is filtered and freed from inorganic antimony as much as possible by washing with dilute hydrochloric acid. The compound thus purified is treated with excess of 5*N* sodium hydroxide solution and vigorously stirred with cooling to ensure the maximum yield.

Various methods of purifying the stibinic acids are known but most of them are either wasteful or unsuitable. After many trials, the following method has been adopted for purification of the stibinic acids described in this paper. The crude acid is dissolved in very dilute sodium hydroxide solution and precipitated by very dilute hydrochloric acid ; this is repeated several times, the acid being allowed to settle and separated by decantation. Coagulation of the colloidal acids is easily effected by saturating the solution with common salt. Finally, the acid is filtered, washed thoroughly with water and the adsorbed inorganic matter removed by shaking repeatedly with a large quantity of water for three or four days.

When the double compound of the aryl-diazoniumchloride and anti- trichloride gives with alkali an insoluble product, this is filtered and wash- h water and extracted thoroughly with a large quantity of glacial acetic warming the mixture is not harmful as decomposition is very slight below *The extract is poured drop by drop into a large quantity of very dilute* nia, when an amorphous precipitate is formed and is further purified by ing the process. Thorough washing with ether gives finally a pure : of the distibinous oxide.

Two of the best methods available for the estimation of antimony are of Ghosh (*Ind. J. Med. Res.*, 1928, **16**, 458) and Macallum (*J. Soc. Chem.* 1923, **42**, 470). Ghosh's method is the safer, although tedious, and the agree very well. In a few cases, confirmatory estimations were carried Macallum's method and the results found to be quite satisfactory, care always taken to decompose the organic matter very slowly.

m-*Phenylenedistibinic acid* (I).—A mixture of *m*-phenylenediamine chloride (10 g.) and antimony chloride (28 g.) was diazotised and the dark ouble salt treated with alkali. A dark red solution was obtained when ion of nitrogen ceased, and on acidification gave a dark brown, colloidal itate which was purified in the usual manner (yield, 5 g.). The acid is ble in hydrochloric acid but is easily soluble in alkalis (Found: Sb, $C_6H_8O_6Sb_2$ requires Sb, 58.1 per cent.).

)-*Phenylenedistibinic acid* (II).—The double salt was dark grey and dark- exposure to air (Found: Sb, 57.8; $C_6H_8O_6Sb_2$ requires Sb, 58.1 per

: 4-*Naphthylenedistibinic acid* (III).—1 : 4-Naphthylenediamine sul- 'as prepared by the method of Griess (*Annalen*, 1866, **137**, 60). A of the diamine sulphate (5 g.) and antimony trioxide (8 g.) in hydro- cid (50 c.c.) was carefully diazotised and the resulting yellow double d treated with 5*N* sodium hydroxide solution. The greenish brown acid obtained on acidification (yield, 0.5 g.) was purified in the usual (Found: Sb, 50.8; $C_{10}H_{10}O_6Sb_2$ requires Sb, 51.9 per cent.).

iphenylene-pp-*distibinous oxide* (IV).—A mixture of benzidine (20 g.) lution of antimony trioxide (30 g.) in concentrated hydrochloric acid otised with sodium nitrite (10 g.). The double salt was soluble in ated hydrochloric acid from which it was precipitated by water as a ellow, crystalline powder; alkali treatment gave an insoluble orange- oduct which on extraction became a light yellow powder (5 g.). Con- l hydrochloric acid produces an intense violet colouration which dis- on dilution with water (Found: Ghosh; Sb, 57.9, 57.5, 57.3, 57.8, acallum; Sb, 58.1, 57.3; mean 57.54; C, 29.95; H, 1.91. $C_{12}H_8O_2Sb_2$ Sb, 56.96; C, 30.1; H, 2.09 per cent.).

)-*Distibinotetraphenylene* (IV *a*).—A small quantity of the finely l diphenylene-*pp*-distibinous oxide was heated with aqueous sodium ate solution on the water bath for three hours when the colour changed d; the filtered product was washed with carbon bisulphide to remove sulphur, but isolation of the pure distibino-compound was extremely Hydrochloric and glacial acetic acids decomposed it, the results is being consequently variable.

3 : 3-*Dimethyldiphenylene-4 : 4'-distibinous oxide* (V).—A solution of *o*-tolidine (5 g.) and antimony trioxide (8 g.) in hydrochloric acid was tetrazotised, and the yellow double salt treated with 5*N* sodium hydroxide solution : after vigorous evolution of nitrogen a brown insoluble residue remained. Extraction with glacial acetic acid gave an orange powder (3 g.) which became a brilliant violet with hydrochloric acid (Found : Sb, 53.4 ; $C_{14}H_{12}O_2Sb_2$ requires Sb, 53.4 per cent.).

Distibinotetramethyltetraphenylene.—A small quantity of the distibinous oxide (V) was finely powdered and heated on the water bath with an aqueous solution of sodium thiosulphate for two hours, when the colour changed from orange to brilliant red. The substance was filtered, washed with water, alcohol and then with carbon disulphide to remove all traces of sulphur, but the substance was found on analysis to be impure. It is insoluble in all organic solvents and is decomposed by mineral acids, liberating inorganic antimony instead of becoming violet.

3 : 3'-*Dimethoxydiphenylene-4 : 4'-distibinous oxide* (VI), prepared from dianisidine in the usual manner was obtained after extraction with glacial acetic acid as an orange powder (2 g.), and gave a violet colouration with hydrochloric acid (Found : Sb, 49.5 ; $C_{14}H_{12}O_4Sb_2$ requires Sb, 49.9 per cent.).

*Diphenylmethane-*pp-*distibinous oxide* (VII).—*pp*-Diaminodiphenylmethane was prepared according to the method of Kaufler and Borel (*Ber.*, 1907, **40**, 3254), the 2 : 4- and 4 : 4-isomerides being separated by the method of King (*J.C.S.*, 1920, **117**, 988). The diamine was diazotised in presence of antimony trioxide dissolved in hydrochloric acid and the deep yellow double salt on alkali treatment gave a product insoluble in alkali. The glacial acetic acid extract was red, yielding the distibinous oxide as a light yellow powder (yield, about 5 per cent.). It dissolved in concentrated hydrochloric acid giving a red solution from which the distibinous oxide could be precipitated by ammonia (Found : Sb, 55.0 ; $C_{13}H_{10}O_2Sb_2$ requires Sb, 55.2 per cent.).

*Benzophenone-*pp-*distibinic acid* (VIII).—*pp*-Diaminobenzophenone was prepared from *pp*-diaminodiphenylmethane by the method of Rivier and Farine (*Helv. Chem. Acta*, 1929, **12**, 866). Stibinated as in the case of *pp*-diaminodiphenylmethane, the bright yellow double salt on treatment with alkali gave a scarlet solution which on acidification gave the brown distibinic acid (yield, about 50 per cent.). The free acid does not show any colouration with hydrochloric acid and is quite stable when dry (Found : Sb, 46.2 ; $C_{13}H_{12}O_7Sb_2$ requires Sb, 46.5 per cent.).

*Diphenyl-*m-*tolylmethylcarbinol-*ppp-*tristibinic acid* (IX).—Rosaniline (5 g.) was diazotised and combined with antimony trichloride, the orange double salt being treated with alkali under ice-cooling ; a red solution of the sodium salt was obtained from which the free tristibinic acid was precipitated as a brown colloidal mass (1.5 g.). The usual method of purification was employed (Found : Sb, 45.9 ; $C_{20}H_{21}O_{10}Sb_3$ requires Sb, 46.5 per cent.).

*Diphenyl-*m-*tolylmethane-*ppp-*tristibinous oxide* (X).—Leucaniline (2 g.) was mixed with the required quantity of antimony trichloride and diazotised. The orange double salt with alkali under ice-cooling gave a very poor yield of an insoluble amorphous brown residue which dissolved in glacial acetic acid

with a deep purple colouration, thus resembling diphenylmethane-*pp*-distibinous oxide (VII). The tristibinous oxide was a dark grey powder (Found : Sb, 55.0 ; $C_{20}H_{15}O_3Sb_3$ requires Sb, 54.6 per cent.).

Stilbene-4 : 4'-distibinic acid (XI) and *stilbene-4 : 4'-distibinous oxide* (XII).—*pp'*-Diaminostilbene was prepared from the *pp*-dinitro compound by reduction with tin and hydrochloric acid (Horman, *J. pr. Chem.*, 1889, ii, **39**, 502), the dinitro-compound having been prepared in its turn from *p*-nitrobenzyl chloride (*Ber.* 1876, **6**, 328 ; 1890, **23**, 1959). The dinitro-compound melted at 281°, not at 280-285° as stated by previous workers, and *pp*-diaminostilbene melted at 226-227°.

Preparation of the antimony compounds.—The diamine (2 g.) was mixed with antimony trichloride dissolved in concentrated hydrochloric acid (30 c.c.) and diazotised. The bright yellow double salt purified in the usual manner was treated with 5*N* sodium hydroxide solution under ice-cooling. The brownish red filtrate gave on acidification a colloidal distibinic acid (0.2 g.) which did not develop the violet colouration (Found : Sb, 45.8 ; $C_{14}H_{14}O_6Sb_2$ requires Sb, 45.8 per cent.).

In this reaction, besides the brown filtrate a large quantity of an insoluble product was obtained and extracted with glacial acetic acid giving a pale brown distibinous oxide (0.4 g.) with ammonia (Found : Sb, 54.3 ; $C_{14}H_{10}O_2Sb_2$ requires Sb, 53.6 per cent.). The quantity of the distibinic acid was much less than that of the distibinous oxide and the conditions of experiment did not seem to have much effect on the yield of the distibinic acid. The diamino-compound used was the one described in literature (m.p. 226-227°) and no attempt was made to prepare the antimony compound by Schmidt's modification of Bart's method.

The double salt, $CO(NH.C_6H_4.N_2Cl,SbCl_2,HCl)_2$.—*sym-pp*-Diaminodiphenylurea was prepared by the improved method of Mistry and Guha (*J. Ind. Chem. Soc.*, 1930, **7**, 793). The diamine (4.5 g.) was diazotised and combined with antimony trichloride, the bright yellow double salt being washed repeatedly with dilute hydrochloric acid and with water, and then dried in a vacuum desiccator over calcium chloride (Found : Sb, 25.9 ; $C_{13}H_{12}ON_6Cl_{10}Sb_2$ requires Sb, 28.0 per cent.).

The secondary monostibinic acid (XIII).—The foregoing double salt was treated with alkali and when the evolution of nitrogen had ceased the reaction mixture was warmed on the water bath for a few minutes and then filtered. The residue was extracted repeatedly with warm dilute alkali until no more dissolved, the alkaline extract on acidification giving a brown colloidal product (1.0 g.), sparingly soluble in alkali and coloured deep violet by hydrochloric acid. Samples of different preparation were identical in appearance and gave concordant results of analysis (Found : Sb, 32.9 ; C, 42.4 ; H, 2.9 ; $C_{13}H_{11}O_3N_2Sb$ requires Sb, 33.3 ; C, 42.8 ; H, 3.0 per cent.).

The same *monostibinic acid* (XIII) from *p*-aminodiphenylurea.–The amine was prepared according to the method of Mistry and Guha (*loc. cit.*), and the method of preparing the antimony compound was the same as in the foregoing experiment (Found : Sb, 33.1 ; C, 42.4 ; H, 3.1). The product (0.5 g.) was identical in appearance and chemical behaviour with the antimony compound obtained from *pp*-diaminodiphenylurea.

4 : 4'-*Diacetylaminodiphenylmethylenediamine.*—Although known in the literature, no details are given for its preparation. A solution of acetyl-*p*-phenylenediamine (12 g.) and methylene bromide (3.5 g.) in amyl alcohol was heated under reflux for three hours. The solid formed on cooling was washed with water and boiled with alcohol; the grey, insoluble product (m.p. 220°, yield 2 g.) was hydrolysed by boiling with hydrochloric acid and the resulting solution on evaporation gave the desired dihydrochloride. The quantity of acetyl-*p*-phenylenediamine should always be in sufficient excess for absorbing the hydrochloric acid formed in this reaction as otherwise this might cause premature hydrolysis. The yield is very low but the method is convenient.

Methylenediaminophenylene-4 : 4'-*Distibinous oxide* (XIV).—Diaminodiphenylmethylenediamine hydrochloride (2 g.) was tetrazotised and combined with antimony trichloride, the bright yellow double salt being treated with alkali in the usual manner. The brown, alkali-insoluble residue in very poor yield, was purified by extraction with glacial acetic acid, when the pale brown powder gave a violet colouration with hydrochloric acid (Found : Sb, 51.2 ; $C_{13}H_{12}O_2N_2Sb_2$ requires Sb, 51.6 per cent.).

4 : 4'-*Diacetylaminodiphenylethylenediamine.* — This compound was prepared from ethylene dibromide (1 mol.) and acetyl-*p*-phenylenediamine (4 mol.), the purified product melting with decomposition at 284° (Found : N, 14.0 ; $C_{18}H_{22}O_2N_4$ requires N, 14.11 per cent.). The diacetyl compound on hydrolysis gave the hydrochloride which was used for stibination.

Ethylenediaminodiphenylene-pp-*distibinous oxide* (XV).—The deep yellow antimony chloride double salt produced an insoluble brown mass on alkali treatment ; the glacial acetic acid extract gave when poured into ammonia, a very poor yield of the brownish distibinous oxide showing the violet colouration with hydrochloric acid (Found : Sb, 50.4 ; $C_{14}H_{14}O_2N_2Sb_2$ requires Sb, 50.2 per cent.).

Bis-p-*aminophenylazomethine.*—Glyoxal sodium bisulphite (5 g.) was warmed with 25 c.c. of water and 3 or 4 drops of sulphuric acid for four hours, a little more acid being added at the later stage of the decomposition, when a clear solution of the free glyoxal was obtained. This solution, together with the required quantity of an alcoholic solution of acetyl-*p*-phenylenediamine, was heated under reflux for an hour when crystals began to separate. The crystals were filtered, and the filtrate yielded two more crops on concentration, the assembled product crystallising from alcohol in silvery plates, m.p. 279° (decomp.). The free *base* was prepared by hydrolysing the diacetyl compound with aqueous-alcoholic hydrochloric acid on the water bath till the reaction mixture left no insoluble residue on being diluted with water. The solution on being treated with dilute ammonia deposited silvery crystals of the free diamine m.p. 258° (decomp.) (Found : N, 17.31 ; $C_{18}H_{18}O_2N_4$ requires N, 17.28 per cent.).

Bis-phenylazomethine-pp'-*distibinous oxide* (XVI).—The free base (2 g.) mixed with antimony trichloride and hydrochloric acid was diazotised, the orange yellow double salt on being treated with alkali under ice-cooling giving a very poor yield of a reddish brown powder which on extraction with glacial acetic acid became a dirty red (Found : Sb, 49.1 ; $C_{14}H_{10}O_2N_2Sb_2$ requires Sb, 50.57 per cent.).

sym-*Oxalaminodiphenylene*-pp-*distibinic acid* (XVII).—*sym-pp*-Diaminodiphenyloxamide was prepared according to the method of Mistry and Guha (*loc. cit.*). The orange coloured double salt obtained from the diamine by diazotisation and combination with antimony trichloride was treated with alkali under ice-cooling and stirring, the solution giving on acidification an orange-grey colloidal precipitate (yield, about 35 per cent.) which was further purified. The dry acid is not very stable but the slightly alkaline solution can be kept for a very long time without appreciable decomposition (Found : Sb, 41.9 ; C, 28.1 ; H, 2.8 ; $C_{14}H_{14}O_8N_2Sb_2$ requires Sb, 41.88 ; C, 28.9 ; H, 2.4 per cent.).

Malonaminodiphenylene-pp-*distibinic acid* (XVIII).—The *sym-pp*-diaminodiphenylmalonamide prepared according to the method of Mistry and Guha (*loc. cit.*), was diazotised and combined with antimony trichloride to a light orange coloured double salt, giving on treatment with alkali a dark-red solution from which acids precipitate the product (yield, about 50 per cent.). The distibinic acid in slightly alkaline solution is quite stable, but in the dry state tends to decompose ; it gives with strong hydrochloric acid a violet colouration which disappears on dilution (Found : Sb, 41.2 ; C, 30.4 ; H, 2.2 ; $C_{15}H_{16}O_8N_2Sb_2$ requires Sb, 40.88 ; C, 30.2 ; H, 2.7 per cent.).

Succinaminodiphenylene-pp-*distibinic acid* (XIX).—*sym-pp*-Diaminodiphenylsuccinamide (Mistry and Guha, *loc. cit.*) gave a deep orange antimony double salt which on alkali treatment and subsequent acidification yields (about 50 per cent.) a brown colloidal acid (Found : Sb, 40.1 ; C, 30.9 ; H, 2.9 ; $C_{16}H_{18}O_8N_2Sb_2$ requires Sb, 40.0 ; C, 31.3 ; H, 3.0 per cent.).

sym-*Carbohydrazinodiphenylene*-pp-*distibinic acid* (XX).—*pp'*-Diaminodiphenylcarbohydrazide, prepared from the corresponding dinitro-compound (Mistry and Guha, *loc. cit.*) by reduction with zinc dust and acetic acid, was tetrazotised and combined with antimony chloride. The bright orange double salt on alkali treatment and subsequent acidification gave a dark brown colloidal precipitate (yield, about 30 per cent.) which was further purified. In all its properties it resembled the previously described distibinic acids (XVII-XIX), except that the dry acid decomposed much more rapidly than the other compounds (Found : Sb, 41.6 ; C, 26.4 ; H, 2.8 ; $C_{13}H_{16}O_7N_4Sb_2$ requires Sb, 41.6 ; C, 26.7 ; H, 2.74 per cent.).

sym-pp'-*Diaminodiphenylhydrazodicarbonamide*.—A mixture of hydrazodicarbonamide (1 mol.) prepared according to Thiele (*Annalen*, 1892, **271**, 128) and acetyl-*p*-phenylenediamine (2 mol.) was heated with amyl alcohol under reflux till the evolution of ammonia, vigorous at first, came to an end. The solid was extracted with alcohol, boiled with dilute hydrochloric acid for one hour, and the extract freed from unchanged hydrazodicarbonamide by filtration. The filtrate on evaporation to dryness gave the hydrochloride as violet plates. The free base, liberated by sodium acetate, is greyish-blue, m.p. above 370° ; yield, 40 per cent. (Found : N, 27.92 ; $C_{14}H_{16}O_2N_6$ requires N, 28.0 per cent.).

sym-pp'-*Diacetyldiaminodiphenylazodicarbonamide* was obtained and purified as in the foregoing case by the action of azodicarbonamide with acetyl-*p*-phenylenediamine (m.p. above 370° ; yield, 40 per cent.). The free base was deep blue and did not melt at 350° (Found : N, 27.8 ; $C_{14}H_{14}O_2N_6$ requires N, 28.1 per cent.).

Hydrazodicarbonaminodiphenylene-pp-*distibinic acid* (XXI).—The yellow double salt obtained in the usual way was treated with alkali under ice-cooling, and the brown solution on acidification gave a very poor yield of the brown colloidal distibinic acid (Found : Sb, 39.9 ; $C_{14}H_{16}O_8N_4Sb_2$ requires Sb, 39.8 per cent.).

Hydrazobenzene-pp′-*distibinous oxide* (XXII).—*pp*-Diaminohydrazobenzene was prepared from the azo-compound which in its turn was prepared by the method of Noelting and Binder (*Ber.*, 1887, **20**, 3015). The reduction of the azo-compound was effected by alcoholic ammonium sulphide, and the hydrazo-compound after crystallisation from alcohol melted at 144°. The hydrazo-compound was mixed with antimony trichloride and diazotised, the brownish yellow double compound on alkali treatment giving a dark brown, insoluble product, the glacial acetic acid extract of which gave the distibinous oxide (also in very poor yield) as a brown amorphous powder (Found : Sb, 54.5 ; $C_{12}H_{10}O_2N_2Sb_2$ requires Sb, 53.2 per cent.).

p-*Nitrobenzoyl*-p′-*acetylaminoanilide*, $NO_2.C_6H'_4.CO.NH.C_6H_4.NHAc$.—A mixture of *p*-nitrobenzoyl chloride (12 g.) and acetyl-*p*-phenylene diamine (7 g.) was heated in a hard glass tube at 125° for two hours, the product being extracted several times with dilute sodium hydroxide solution until a sample ceased to give a precipitate of nitrobenzoic acid with hydrochloric acid. The greenish yellow residue after being washed with alcohol, in which it was completely insoluble, was crystallised from pyridine, m.p. 293° (decomp.). Hydrolysis and reduction was effected by heating it on the water bath for two hours with stannous chloride and hydrochloric acid. Tin was removed from the filtered solution by hydrogen sulphide and the clear solution after removal of stannous sulphide gave the hydrochloride on evaporation.

Benzanilide-pp′-*distibinous oxide* (XXIII).—The hydrochloride (2 g.) was mixed with antimony trichloride and diazotised, the yellow double salt on alkali treatment giving an insoluble brown product in very poor yield. Extraction with glacial acetic acid left the pure distibinous oxide (Found : Sb, 50.7 ; $C_{13}H_9O_3NSb_2$ requires Sb, 51.8 per cent.).

Triphenylphosphine-mmm-*tristibinic acid* (XXIV).—Triphenylphosphine was prepared according to the method of Pfeiffer, Heller and Pitsch (*Ber.*, 1904, **37**, 4621) giving with the following modification a better yield. Addition of the ethereal solution of phosphorus trichloride to phenyl magnesium bromide was made under ice-cooling during four hours. The product, m.p. 78°, was obtained in 95 per cent. yield.

Nitration of triphenylphosphine was effected by the method of Michaelis and Soden (*Annalen*, 1885, **229**, 324). The nitrated product tended to form a sticky mass which, however, on being triturated with an aqueous solution of urea became quite loose and flocculent and after crystallisation from acetic acid melted at 241-242°. Michaelis and Soden regarded it (m.p. 242°) as a *para*-substitution product, but Challenger and Wilkinson showed it to be the *meta*-derivative (*J. C. S.*, 1924, **125**, 2675).

Reduction of the trinitro-compound was conducted by heating it on the water bath with zinc dust and acetic acid. On dilution with water and addition of alkali the precipitate of triamine was practically pure and was crystallised from alcohol, m.p. 255°. A hydrochloric acid solution of *mmm*-triaminotriphenylphosphine (5 g.) and antimony trioxide (7 g.) was diazotised

with sodium nitrite (3 g.) and the yellow double salt thus obtained treated with 5*N* alkali under ice-cooling. The brown solution on acidification yielded the colloidal tristibinic acid which required nearly two days to settle, but addition of common salt hastened the process of coagulation considerably; it was further purified in the usual manner.

Estimation of antimony was effected by mixing the substance (about 0.2 g.) with concentrated sulphuric acid (10 c.c.) and anhydrous potassium sulphate (3 g.), then heating the mixture very gently and cautiously till the solution became colourless. On dilution with water, 15 c.c. of hydrochloric acid was added and the antimony sulphide precipitated with hydrogen sulphide was dissolved in very dilute sulphuric acid and oxidised with potassium permanganate, excess of which was removed by boiling with hydrochloric acid; the colourless solution was cooled, mixed with potassium iodide (2 g.) and titrated against standard thiosulphate using starch as an indicator (Found: Sb, 46.8; $C_{18}H_{18}O_9PSb_3$ requires Sb, 47.2 per cent.).

Triphenylarsine-mmm-*tristibinic acid* (XXV).—Triphenylarsine was prepared in the same manner as the phosphine, nitration and reduction being carried out by the method of Michaelis (*Annalen*, 1902, **321**, 180). A mixture of the hydrochloric acid solution of the triamine and the required quantity of antimony trichloride on diazotisation gave a yellow double compound which on treatment with 5*N* alkali under efficient ice-cooling gave a brown solution from which the colloidal acid was liberated and purified in the usual manner. Estimation of arsenic and antimony was conducted according to the modified method of Fargher (*J. C. S.*, 1919, **115**, 992. Found: Sb+As, 54.28; $C_{18}H_{18}O_9AsSb_3$ requires Sb+As, 53.8 per cent.).

Triphenylstibine-mmm-*tristibinic acid* (XXVI).—Triphenylstibine was prepared by the method described in Organic Synthesis, Vol. VII, 80, while the nitration and reduction were effected by the method of Morgan and Micklethwait (*J. C. S.*, 1911, **99**, 2299). The yellow double salt on alkali treatment gave a brown solution which on acidification gave the tristibinic acid which did not show the colour reaction of the distibinic acids (Found: Sb, 55.9; $C_{18}H_{18}O_9Sb_4$ requires Sb, 56.3 per cent.). The tristibinic acids here described are all very unstable and in the dry state decompose very easily.

Summary.

With a view to throwing some further light on the chemotherapy of organic antimonials a large number of aromatic di- and polyamines having the amino-groups attached to the same or different phenyl radicals have been stibinated according to the well-known method of Bart and Schmidt. Contrary to the general rule that aromatic amines on stibination yield only the corresponding stibinic acids, quite a large number of di- and polyamines have now been found to yield di- and poly-stibinous oxides (IV-VII, X-XII, XIV-XVI, XXII, XXIII). The distibinous oxides are not affected by mild oxidising agents such as hydrogen peroxide, and are decomposed when subjected to more drastic processes of oxidation. On reduction, however, they give rise to highly coloured products which could not be sufficiently purified owing to their unstable nature, but are probably distibino-compounds.

In one case, namely, *pp*-diaminodiphenylurea, a compound has been obtained which in all probability is a secondary monostibinic acid (XIII).

The work is being continued, and although it is not possible at this stage to formulate any definite rule underlying the formation of stibinous oxides and stibinic acids, it can, however, be said that—

(1) *pp*-Amino-groups in diphenyl give stibinous oxides.

(2) The groups CO and C(OH) connecting two or more phenyl radicals aid in converting *p*-amino-compounds into stibinic acids, whereas the CH_2 group favours the formation of stibinous oxides.

(3) Connecting links involving nitrogen besides carbon, when oxygenated, also yield stibinic acids, whereas in absence of oxygen the stibinous oxides are produced. Only one exception to this rule, namely, diaminobenzanilide, which yields the distibinous oxide, has yet been observed.

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BHADRAVATI WOOD-TAR AND ITS UTILIZATION.

By Y. K. Raghunatha Rao, B. Sanjiva Rao and H. E. Watson.

I. COMPOSITION OF THE TAR.

The tar obtained by the distillation of hardwood is known to be a mixture of extraordinary complexity, and detailed investigations of its constituents are few in number. Among the most noteworthy are those of Behal and Choay (*Compt. rend.*, 1893, **116**, 197; 1896, **119**, 166) who found phenol, the three cresols, *o*-ethylphenol, 1:3:4- and 1:3:5-xylenols, guaiacol, creosol and ethylguaiacol in the tar from beech and oak wood, and of Pieper, Acree and Humphrey (*Ind. Eng. Chem.*, 1917, **9**, 462) who found the dimethyl ethers of pyrogallol, methylpyrogallol and propylpyrogallol in the higher fractions of maple tar. Coerulignol was observed by Pastrovitch (*Monatsh.*, 1883, **4**, 188) in beech tar, homopyrocatechol dimethyl ether was separated by Tiemann and Mendelsohn (*Ber.*, 1875, **8**, 1136) from the same material, while more recently, Williams, Lasselle and Reed (*Ind. Eng. Chem.*, 1925, **17**, 85) have described the detection of pyrogallol monomethyl ether.

The products of the Mysore wood distillation plant at Bhadravati differ from those commonly obtained in commerce in that they are derived from more than 50 species of woods, as opposed to the one or two usually utilized. The tar is thus likely to be even more complex in character than normal tar, but at the same time the possibility of finding unusual constituents seemed to render an examination worth while and consequently the tar has been separated as far as possible into its components.

Most of the experiments were made with a 5-gallon sample of "refined tar" drawn from the bulk supply. This consisted of the tar settled from the pyroligneous acid after heating to expel most of the water and acetic acid, a small quantity of light oil being removed simultaneously.

Physical Properties.

The more important physical properties of this tar are given in Table I, and for comparison a specification of the British Road Tar Association for a tar suitable for use on roads is adjoined. The viscosity was determined by the modified Redwood viscometer (*cf.* Butterfield, *J.S.C.I.*, 1928, **47**, 298T), free carbon by extraction with toluene, and phenols by treatment with sodium hydroxide.

TABLE I.

Properties of Refined Tar.

	Wood Tar	B. R. T. A. Tar No. 2
Specific gravity	1.156 (24°)	1.150—1.240
Viscosity at 30° (Mod. Redwood)	45 secs.	40—125 secs.
Flash point (Pensky-Marten) ..	104°	....
Continuous fire point ..	135°	....
Water	0.6 per cent.	0.5 per cent.
Distillate below 200°	1.5 ,,	1.0 ,,
,, 200–270° ..	36.6 ,,	8.0–16.0 ,,
,, 270–300° ..	Trace. ,,	3.5–12.2 ,,
Phenols and acids	21.9 ,,	4.0 ,, (vol.)
Naphthalene	*Nil.*	5.0 ,,
Free carbon	17.0 ,,	6–21 ,,
Ash	0.5 ,,	....

It is well known that wood tar is not a suitable material for tarring roads and the sample under discussion is no exception, owing to the high proportion of comparatively low-boiling oil and the absence of an intermediate fraction between this and the pitch.

Solvent Extraction.

In order to obtain a general idea of the nature of the tar, portions were extracted with organic solvents and the solutions examined: 250 c.c. were treated with light petroleum (b.p. 40–60°) and the insoluble portion further extracted with benzene. Two samples of 20 c.c. were separately treated with ether and benzene. The petroleum extract was shaken with 10 per cent. sodium hydroxide solution and then with 25 per cent. acetic acid to remove acidic and basic bodies; the neutral oils obtained after washing and removing the solvent were distilled. The phenols and acids were recovered from the sodium hydroxide solution by acidifying with 10 per cent. sulphuric acid after a preliminary washing with ether to remove neutral bodies and extraction with ether. A small quantity of tar bases was obtained from the acetic acid solution by adding 40 per cent. sodium hydroxide solution and extraction with ether. An analogous procedure was followed in the case of the other solvents.

For comparison, 20 g. of tar were treated directly with 10 and 20 per cent. sodium hydroxide solutions, the aqueous solution acidified after shaking with ether, and the neutral bodies taken up in ether and filtered from the solids. Table II gives a resume of the results obtained.

TABLE II.

Extraction of Tar.

	Solvent	Petrol	Benzene	Petrol & benzene	Benzene	Ether	NaOH 20%	NaOH 10%
(*a*)	Total extract	35.4	44.4	79.8	87.1	84.2	68.8	70.0
(*b*)	Solid residue	..	18.7	18.7	12.8	15.8	11	10
(*c*)	Acidic bodies	10.1	33.3	43.4	..	49.2	69	70
(*d*)	Neutral oils	24.7	10.1	34.8	..	35.0	20	20
(*e*)	Resin on distilling (*c*)	0.8	14.3	15.1	..	19.2	39	34

The petroleum ether extract contained 0.66 per cent of bases. The neutral oils obtained from this solvent boiled at 160–300°, had a density of 0.817 at 24° and n_D^{24} 1.4820. Those insoluble in this solvent but soluble in benzene had a similar range of boiling point, but the refractive index was 1.5000. The acidic bodies soluble in petroleum ether boiled from 180 to 270° at atmospheric pressure and those obtained directly with sodium hydroxide between the same temperatures. The constituents of the two liquids were, however, very different, the former distilling almost completely and yielding a distillate with density 1.06 while the latter, when about half had been distilled, left a resinous mass which decomposed on further heating. The amount of residue calculated for 100 g. of original tar is shown in row (*e*). The reason for this difference in behaviour appears to be the presence of ulmins and rhetinols which together with other neutral substances dissolve very readily in aqueous sodium phenate solution, and are not easily removed by washing

with ether. If sodium hydroxide is added to a solution of the phenol in benzene or ether the sodium phenate is less contaminated than it is when no solvent is present (*cf.* Edwards, *J.S.C.I.*, 1924, **43**, 143T; Parish and Rowe, *ibid.*, 1926, **45**, 99T).

A second factor is probably the decomposition of some of the neutral oils by the aqueous sodium hydroxide in absence of a solvent giving rise to substances partly or wholly soluble in alkali. Experimental evidence on this point is not definite, but if some such action does not take place it is difficult to explain why the aqueous solution after washing with ether should contain as much as 20 per cent. more soluble matter than a solution obtained by adding sodium hydroxide to the ethereal extract.

On distilling the phenolic bodies obtained by means of aqueous sodium hydroxide either alone or in presence of an organic solvent, a distillate equivalent to about 30 per cent. of the original tar is obtained, whereas if the tar itself is distilled and the distillate extracted with sodium hydroxide solution (see next section) the yield of phenols is only 22 per cent. The former procedure would appear preferable, but in practice it is difficult to follow owing to the formation of emulsions. The yield of neutral oils is, as may be seen from Table II, much larger when a solvent is used, being 35 per cent. as compared with 20 per cent. for sodium hydroxide alone. If the tar is distilled before extraction this figure is reduced to 15 per cent.

Distillation of Tar.

At first, 500 g. portions of the tar were distilled from a glass distilling flask at atmospheric pressure (680 mm.), so that the course of the distillation might be watched. Subsequently a 3-litre iron retort heated in a fusible metal bath was employed enabling 1500 g. to be treated at once. It was found that at atmospheric pressure the temperature of the vapour rose slowly as distillation proceeded until about 220°, when it became irregular, sometimes rising to 260° and sometimes falling as low as 200° owing to decomposition of the higher boiling portions. By continuing the distillation until the distillate practically ceased, it was possible to obtain 36–37 per cent. of a yellow oil. At reduced pressure there was a steady temperature rise and little or no decomposition. Table III shows some of the results obtained.

TABLE III.

Yield of Tar Distillate at Different Pressures.

Pressure mm.	100	115	135	150	150	160	680
Max. temperature	153	164	170	170	170	170	260
Distillate per cent.	30.4	33.1	33.2	35.0	34.0	34.7	37.3

The temperatures at which distillation started were somewhat indefinite owing to the presence of small quantities of water, but were in the neighbourhood of 130° at 150 mm.

Separation into Constituent Groups.

Extraction of Acids.—1750 g. of the crude distillate were redistilled at 150 mm. pressure and the product shaken in two batches with 250 c.c.

of 10 per cent. sodium carbonate solution for half an hour. The aqueous solution was separated and washed with ether and the acids liberated with sulphuric acid and extracted with ether.

Extraction of Phenols.—The residual oil from the last operation was treated with 1250 c.c. of 20 per cent. sodium hydroxide solution and ether added. Some sparingly soluble sodium salts were dissolved in the minimum quantity of water and the whole of the aqueous liquid well washed with ether. Addition of ether precipitated a small quantity of a dark brown solid which was probably a mixture of resinols. The phenols were liberated by sulphuric acid, redissolved and reprecipitated, the aqueous solutions being extracted with ether.

Extraction of Bases and Neutral Oils.—The ethereal solution of oils insoluble in sodium hydroxide solution was shaken with excess of sulphuric acid, the aqueous solution made alkaline and extracted with ether yielding 8 g. of bases. The remaining neutral oils were freed from ether and distilled.

The Acids.

Acids (32 g.) were obtained representing 1.66 per cent. of the tar distillate. 10 g. were steam distilled and silver salts prepared from the four fractions in which the distillate was collected. The equivalents of the acids in each fraction were found to be 93.8, 100.2, 105.6 and 106.8, indicating that they might be mixtures of butyric (88) and valeric (102) acids with small quantities of higher acids.

After a preliminary fractionation at 35 mm., 20 g. of the acids were refractionated at a pressure of 27 mm. with the results shown in Table IV, the refractive index and equivalent of each fraction being determined, the latter by titration.

TABLE IV.

Fractionation of Acids : 20 g., 27 mm.

Fraction	Temp.	Distillate g.	n_D^{30}	Equivalent	Acid	n_D^{30}	Equivalent
1.	55— 60	0.5	1.3825	*n.d.*	C_3	1.383	74
2.	60— 70	1.5	1.3880	80.4			
3.	70— 80	1.0	1.3970	79.4			
4.	80— 90	1.0	1.4055	88.0	C_4	1.393	88
5.	90—100	0.5	1.4100	96.5			
6.	100—105	2.0	1.4200	95.0			
7.	105—110	2.0	1.4345	98.4	C_5	1.400	102
8.	110—115	1.0	1.4415	112.0			
9.	115—120	1.5	1.4500	113.4			
10.	120—125	1.0	1.4610	115.4	C_6	1.410	116
11.	125—130	1.0	1.4780	121.3			
12.	130—135	0.5	1.4905	129.7	C_7	1.417	130

Their general properties such as smell and solubility suggested that these acids might be the lower members of the aliphatic series, the equivalents of which are given in the last column. Comparison of the refractive indices showed however that this was not so in all cases, as the differences were far too great to be accounted for by experimental error. The first three fractions were readily soluble in water but the rest were not, while all except the first

wo smelt very strongly of butyric acid. The later fractions gave a slight ellow colour with ferric chloride and decolorized permanganate slowly in the old. It is thus probable that the fractions 1 to 5 consisted mainly of ropionic and butyric acids.

A review of the refractive indices of all the aliphatic acids with mole-ular weights below 130 revealed none approaching 1.47. Tiglic acid (m.w. 100) vhich has been observed in wood tar has a refractive index of 1.451 and may ave been present in the middle fractions, but it could not be isolated. On recipitating the silver salt from fraction 12 and regenerating the acid, an mpure solid melting at 62–65° was obtained, the quantity being too small for dentification.

It would thus appear that in order to account for the high refractive ndex, aromatic compounds must have been present, and since even benzoic cid has a molecular weight of 122, the low equivalents must have been due o the presence of more than one acidic group. The higher fractions, there-ore, probably contained dibasic aromatic acids, phenolic acids or even the higher henols, since several of these are soluble in sodium carbonate solution. With he quantities available, further separation and identification were not possible.

The Bases.

The bases, 8 g. or 0.41 per cent. of the distilled tar, formed an extremely auseous liquid. They were colourless, lighter than water and miscible with t, the higher boiling fractions only partially. They gave the carbylamine eaction and contained nitrogen. On distillation at atmospheric pressure 1.5 g. vas obtained boiling between 40° and 100°, and 5.5 g. between 100° and 220°. The latter fraction had $n_D^{22}=1.3760$ and $d^{22}=0.984$, and yielded a solid pic-ate melting at 160–165°. Pyridine and its homologues were probably pre-ent, but in view of the obvious complexity of the mixture further investiga-ion with the small quantities available appeared of no value.

The Phenols.

Fractionation.—The phenols isolated as already described and forming 59.7 per cent. of the redistilled tar were submitted to a preliminary fractiona-ion at a pressure of 20 mm. in portions of 400 g. using 3 types of fractionating olumns for comparative purposes. Table V gives the results of 3 distillations.

TABLE V.

Distillation of Phenols: 20 *mm.*

		Widmer			Young 4-bulb		Lebel-Henninger 3-bulb		
Fraction	Temp.	Dist.%	n_D^{30}	d_{30}^{30}	Dist.%	n_D^{30}	Dist. %	n_D^{30}	d_{30}^{30}
A	100–110	29.5	1.5300	1.064	6.2	1.5295	8.7	1.5290	1.064
B	110–120	16.7	1.5280	1.055	29.1	1.5305	30.7	1.5305	1.064
C	120–130	7.6	1.5250	1.046	13.5	1.5290	12.4	1.5280	1.061
D	130–140	4.5	1.5274	1.058	14.5	1.5308	6.8	1.5285	1.071
E	140–150	6.1	1.5400	1.115	6.2	1.5355	8.5	1.5360	1.096
F	150–160	13.6	1.5455	1.145	10.9	1.5440	19.7	1.5450	1.139
G	160–170	4.5	1.5435	1.137	7.5	1.5460			
H	170–180	3.8	1.5330	1.116	2.6	1.5470	3.4	1.5480	1.129

The Widmer column (*Helv. Chim. Acta*, 1924, **7**, 59) appeared to give the best separation and was used in subsequent distillations. Distillation at

atmospheric pressure was found unsatisfactory as there was much resinification and a residue of 14.7 per cent., more than twice the amount under reduced pressure.

Fractions A and B were mixed and 740 g. were twice refractionated and collected in 12 fractions boiling between 86° and 121°/20 mm. Measurements of refractive index showed a considerable variation in the nature of these fractions. On nitration, picric acid was obtained from the first fractions and tri*nitro-m*-cresol from those boiling between 90° and 103° but attempts to prepare other derivatives with acetic anhydride, chloracetic acid, benzoyl chloride, picric acid and *p*-nitrobenzoyl chloride were unsuccessful. Steam distillation was found of no value for effecting a separation. Qualitative tests showed a fairly regular gradation in properties ; for example, ferric chloride gave a yellow colour and barium hydrate no reaction with the first 7 fractions, while with the remaining 5, red and blue colorations were produced with these reagents. Silver nitrate was not reduced by the first 4 fractions but was acted upon slowly by the rest. It was thus evident that the mixture, consisting probably of mono-, di- and trihydroxyphenols, cresols, xylenols and their partly methylated derivatives was too complex to resolve by fractionation alone. It was therefore decided to demethylate each fraction so that only the phenols would have to be identified.

Demethylation.—Preliminary experiments showed that the fractions boiling up to 130° could be demethylated by heating with concentrated hydrochloric acid in sealed tubes at 120°. The higher-boiling fractions, however, did not yield so readily to this treatment, a prolonged heating period being required, the result of which was resinification. Better results were obtained by boiling the phenol with 6 times its volume of mixed acetic and hydrobromic acids in equal parts for 8 hours (*cf.* Stoermer, *Ber.*, 1908, **41**, 321). The whole of the phenols boiling below 140° were consequently treated by the former method and the remainder by the latter. In both cases the products were poured into water and the phenols extracted with ether and washed with sodium carbonate solution. After removing the solvent the residue was steam distilled and the phenols recovered with ether. This effected a separation of the monohydroxyphenols which are volatile in steam and the polyhydroxy-compounds which are non-volatile. Table VI shows the percentage methoxyl content of the fractions before and after treatment with acid and the approximate composition. Demethylation was not quite complete in the case of the higher fractions but was sufficient to permit of tests for the components of the mixture.

TABLE VI.

Composition of Phenol Fractions.

Fractions	A+B	C	D	E	F	G+H
Initial OMe ..	7.8	6.8	9.0	11.9	16.0	4.0
Final OMe	0.0	0.0	0.6	1.4	2.0	*n.d.*
Monohydroxyphenols per cent. on total phenols.	15.3	4.3	2.2	1.4	1.8	1.0
Polyhydroxyphenols and derivatives ..	16.8	6.3	8.4	4.5	5.8	2.4
OMe in above ..	16.8	14.4	15.5	16.0	18.6	16.7
Decomposed, per cent. ..	3.9	2.8	4.0	0.3	3.8	6.7

The quantities of monohydroxyphenols are those actually found, calculated as percentages on the total phenols. The amounts of polyhydroxyphenols and derivatives are calculated from the quantity of polyhydroxyphenols obtained after demethylation assuming that the whole of the methoxyl in the fraction was originally combined with them. The methoxyl content of these compounds in all the fractions calculated with the same assumption is not far from 17 per cent. indicating that the constituents are mainly monomethoxy-compounds mixed with smaller amounts of polyhydroxyphenols. All the figures are necessarily very approximate as a comparatively large proportion of each fraction decomposed during the operation of demethylation, as shown in the last line of the table.

Identification of Phenols.

(*a*) *Monohydroxyphenols.*—The steam-volatile products obtained after demethylation were separately fractionated at atmospheric pressure and the density and refractive index of each fraction measured. The results are shown in Table VII.

The first column gives the total weight of the original fraction after distillation with the Widmer column. In several cases only a portion was demethylated and steam distilled, but the weights of monophenols are calculated for the whole fraction from the weights actually obtained. In the same way the figures in the last column give the values which would have been obtained had the whole original fraction been demethylated and distilled.

With the exception of the first two fractions which appeared to contain an impurity with low refractive index (possibly small quantities of acids not extracted by sodium carbonate), the physical properties of the lower boiling fractions indicated that they consisted mainly of the three cresols with, perhaps, a little phenol.

Phenol was identified in fractions 1, 2 and 3 by preparation of the benzoate and by nitration to picric acid ; *o*-, *m*- and *p*-cresols were distinguished by condensation with chloracetic acid and fractional crystallisation of the resulting oxyacetic acids from cold benzene (*cf.* Lederer, *D.R.P.*, 79514). The sparingly soluble acid melting at 151–152° corresponding with *o*-cresol (mixed melting point with *o*-cresoxyacetic acid 151°) was found in fractions 2, 3 and 4 ; the more readily soluble derivative of *p*-cresol m.p. 135–136° (mixed m.p. 135°) in fractions 3 and 4, while fractions 4, 5, 6, 7 and 8 yielded the *m*-cresol derivative, m.p. 102° (mixed m.p. 102°; C=65.1, H=6.0 ; $C_9H_{10}O_3$, *m*-cresoxyacetic acid, requires C=64.9, H=6.4 per cent.). Nitration of these fractions gave trinitro-*m*-cresol m.p. 105°, and the benzoate of *p*-cresol was obtained from fractions 3 and 4. It appeared probable that the higher-boiling fractions might contain xylenols, but their identification was a matter of some difficulty owing to the small quantity of material available and the conflicting data found in literature regarding their properties. An indication of those which were present in appreciable quantities was obtained by brominating the mixture in carbon tetrachloride solution, distilling the solvent and leaving the product in the ice chest until crystallisation took place. The crystals were separated and submitted to a number of fractional crystallisations from alcohol. In this way two substances melting at 179–180° and 165° were obtained from

TABLE VII.

Distillation of Steam-Volatile Phenols: 680 mm.

Fraction	Wt. distilled g.	No.	B.P.	d^{30}_{30}	n^{30}_{D}	$FeCl_3$	Wt. of distillate	Percentage on total distilled phenols.
A+B. 461 g. ..	68	1	160–180	1.037	1.4930	Violet.	1	0.3
Monophenols		2	180–185	1.039	1.5250	Blue.	2	0.6
197 g.		3	185–190	1.036	1.5355	,,	9	2.7
		4	190–193	1.033	1.5380	,,	11	3.3
		5	193–195	1.028	1.5370	,,	3	0.9
		6	195–200	1.025	1.5370	,,	9	2.7
		7	200–205	1.018	1.5345	,,	5	1.5
		8	205–215	1.017	1.5320	Bl.-gr.	10	3.0
			Residue				13	3.9
C. 75 g. ..	24.5	9	190–198	1.007	1.5185	Gr.-y.	2	0.2
Monophenols		10	198–200	1.014	1.5275	Blue.	4	0.4
24.5 g.		11	200–205	1.017	1.5300	,,	3	0.3
		12	205–210	1.016	1.5280	Bl.-gr.	4	0.4
		13	210–215	1.018	1.5290	,,	3	0.3
		14	215–220	1.016	1.5280	,,	3.5	0.4
		15	220–225	1.013	1.5280	Gr.-y.	2	0.2
		16	225–230	1.040	1.5290	,,	2	0.2
D. 160 g. ..	15	17	200–210	1.015	1.5210	Yellow.	1.5	0.3
Monophenols		18	210–215	1.016	1.5300	,,	3	0.5
25 g.		19	215–220	1.015	1.5300	,,	2.5	0.4
		20	220–225	1.015	1.5275	,,	2	0.3
		21	225–230	1.018	1.5280	Gr.-y.	1.5	0.3
		22	230–240	1.031	1.5305	,,	1.5	0.3
E. 60 g. ..	5.5	23	210–230	1.02	1.5275	Green.	4	1.2
Monophenols								
16.5 g.								
F. 136 g. ..	8	24	200–240	1.03	1.5290	,,	6	2.4
Monophenols								
31 g.								
G+H. 84 g. ..	3	25	230–240	1.03	1.5305	,,	2	0.4
Monophenols								
6.5 g.								

fraction 13 (b.p. 210–215°) and one melting at 165° from fraction 14. 1:3:4-xylenol and 1:4:5-xylenol boil at approximately 208° and 210° at 680 mm. and their bromides both melt at 179°. A good yield of an oxyacetic acid m.p. 118° which is the melting point of the oxyacetic acid of 1:4:5-xylenol was obtained from fraction 6 (b.p. 195–200°) indicating the probable presence of this isomer and thus it seems likely that the bromide isolated from fraction 13 is also derived from the 1:4:5-compound. Too much attention must not be paid to the discrepancy in boiling points owing to the complexity of the mixtures which were being distilled. The bromide melting at 165° from fraction 13 was readily soluble in alcohol and appeared to be derived from 1:3:5-xylenol (b.p. 216°) the bromide of which melts at 162.5° according to Noelting and Forel (*Ber.*, 1885, **18**, 2679) and at 166° according to Thol (*Ber.*, 1885, **18**, 362). On the other hand the product melting at 165° from fraction 14 was sparingly soluble in hot alcohol and appeared to be the bromide of 1:2:4-xylenol (b.p. 223°), although recrystallisation failed to raise the melting point, which according to Jacobson (*Ber.*, 1878, **11**, 28) should be 169°.

Although the above evidence cannot be regarded as conclusive, it seems highly probable that 1:4:5-, 1:3:5- and 1:2:4-xylenols were among the phenolic constituents, and 1:3:4-xylenol may have been present as well. All attempts to prepare derivatives from the higher fractions have so far failed and thus it is not possible even to indicate their probable composition.

Quantitative estimation.—Attempts were made to estimate the amounts of different phenols in the lower fractions by the method of Redman, Weith and Brock (*Ind. Eng. Chem.*, 1913, **5**, 831) which depends on the formation of di-iodo-compounds by *o*- and *p*-cresol and tri-iodo-compounds by phenol and *m*-cresol. Even when three phenols are present the method is not one of great accuracy, as small experimental errors are largely magnified in the final results; if there are four phenols, or if impurities are present, it is only possible to form a very rough estimate of the total amount of *o*- and *p*-cresol together and of each of the other two. As the quantities of demethylated monophenols after distillation were small, material for this estimation was obtained from fractions A and B distilled with the Lebel column and weighing 170 g. (Table V) which were refractionated at atmospheric pressure and divided into four fractions boiling over the range 180–190° (10 g.), 190–200° (55 g.), 200-210° (60 g.) and 210–230° (45 g.). The results indicated 82 per cent. of *o*- and *p*-cresol and 18 per cent. of phenol in the first fraction and 86 per cent. of *o*- and *p*-cresol and 14 per cent. of *m*-cresol in the second fraction. *m*-Cresol was estimated independently by Raschig's nitration method in the two middle fractions which were found to contain 14 and 6 per cent. Determinations of the amount of phenol in the first fraction were also made by nitration to picric acid, the result being 21 per cent. Calculated as percentages on the total distilled phenols the quantities found by titration are:—phenol 0.42, *m*-cresol 1.8, *o*- and *p*-cresols 13.0 and by nitration, phenol 0.48, *m*-cresol 2.6. Of these values, those for phenol and *m*-cresol are probably low, as not all the nitro-derivatives could be recovered, while the figure for the two remaining cresols is likely to be high since the titration value for *m*-cresol is low.

An approximation to the quantities of cresols and xylenols present can also be obtained from Table VII, taking as a rough guide the colours with ferric chloride which are blue for cresols and blue-green for xylenols, and noting that xylenol derivatives were isolated from fractions 6, 13 and 14. The density

of *o*-cresol at 30° is 1.041, of *p*- and *m*-cresols 1.031 and of the xylenols about 1.017. This enables an estimate to be made of the relative amounts of cresols and xylenols in the rather large fractions 5–8 assuming no other substances to be present. Fractions 1 and 9 appear to contain an impurity with low density and refractive index, possibly acid decomposition products formed on demethylation. Fraction 1 was found to contain 51 per cent. of phenol by nitration. With these premises the quantities of monophenols in the different fractions are as shown in Table VIII.

TABLE VIII.

Quantities of Monophenols as Percentage of Total Phenols.

Fraction	1	2	3	4	5	6	7	8	9	10	11–14	15–17	18–25	Total
phenol ..	0.15	0.35												0.5
o- and *p*-cresol		0.25	2.7	3.1	0.2									6.25
m-cresol ..				0.2	0.5	1.4	0.1	0.1	0.0	0.1				2.4
xylenols ..					0.2	1.3	1.4	2.9	0.05	0.3	1.4	0.3		7.8
other phenols												0.4	5.8	6.2

It will be observed that a considerable proportion of the phenols occurs in the higher boiling fractions, but it has not been found possible to isolate any definite compounds.

(*b*) *Polyhydroxyphenols.*—The non-steam-volatile residue of demethylated phenols from fractions A and B (*cf.* Table VI), after extraction with ether and drying weighed 76 g. This was distilled at 20 mm. pressure and gave 42 g. boiling from 130-160° and 8 g. from 160–200°. The first distillate formed a mass of fine crystals on stirring. The whole was extracted with dry benzene and from the concentrated solution crystals were obtained which after two crystallisations from alcohol melted sharply at 104° and gave all the usual tests for catechol. The bromine derivative melted at 139°. The more soluble portion after several crystallisations from alcohol yielded homocatechol m.p. 52° (m.p. of bromine derivative 162–163°). The formation of catechol after demethylation indicates the presence of guaiacol in the tar. This was further confirmed by the preparation of dinitroguaiacol by addition at zero of fuming nitric acid to the phenol fraction boiling at 113–115°/ 20 mm. (*cf.* p. 46) both in glacial acetic acid solution. It was recrystallised from methyl alcohol and melted at 122° (Found: N, 12.82 ; $C_7H_6O_6N_2$ requires 13.08 per cent.). It was evident that other substances were present but it was not found possible to isolate any of them. The higher boiling fraction, 160-200° formed a jelly-like mass which could not be crystallised from any ordinary solvents.

The higher boiling fractions C-H, Table V, were even more troublesome. After refractionation at 680 mm. of a portion of the distillate boiling at 140–180°/ 20 mm., a fraction boiling at 238–240° was obtained which gave the colour tests for coerulignol, $C_3H_7.C_6H_3(OH).OCH_3$ and contained 16.0 per cent. methoxyl, the value for coerulignol being 18.7. It is probable therefore that this substance, which has been previously found in wood tar (Pastrovitch, *Monatsh.*, 1883, **4**, 188), was present. The fraction 243–244° after demethylation gave the fluorescein test indicating the presence of resorcinol, although

the phenol itself could not be isolated. Resorcinol monomethyl ether (b.p. 240°/680 mm.) appeared to be a likely constituent. The higher fractions gave sodium and potassium salts which became blue in air and deep red on subsequent addition of acid. This reaction is given by pyrogallol and methyl pyrogallol dimethyl ethers and consequently the fraction was demethylated. This caused a considerable amount of resinification and the fraction boiling between 290° and 300° set to a jelly from which it was not possible to obtain crystals. The aqueous alkaline solution, however, rapidly darkened when exposed to air so that pyrogallol was probably present.

In order to form an estimate of the amount of guaiacol, 45 g. of a phenol fraction boiling at 185–215° was redistilled at atmospheric pressure and the methoxyl content of each fraction determined. A higher boiling fraction 220 g. 140–180°/20 mm. was treated in a similar manner. Table IX gives the results and the weight of each fraction.

TABLE IX.

Amount of Methoxy-compounds in Phenol Fractions.

B. P.	Weight g.	OMe per cent.	Guaiacol (202°)	Creosol (217°)
185–190 ..	8	6.1	1.9	
190–195 ..	5	7.4	1.5	
195–200 ..	7	10.3	2.9	
200–205 ..	7	8.0	2.2	
205–210 ..	5.5	7.4	0.8	0.9
210–215 ..	6	11.0	0.4	2.5
			Coerulignol (237°)	Pyrogallol dimethyl ether (249°)
240–245 ..	8	15.6	1.2	2.5
245–250 ..	21	....	1	6
250–255 ..	29	12.8		9.2
255–260 ..	44	10.4		11.5
260–265 ..	34	15.9		13.5
265–270 ..	18	11.8		5.3
270–290 ..	19	....		

Assuming that guaiacol, creosol, coerulignol and pyrogallol dimethyl ether are the only methoxy-compounds present, an approximation to the amount of each may be calculated since their methoxyl values are 25.2, 22.4, 18.6 and 40.2 respectively. The resulting weights in grams are shown in the table, arbitrary values being assigned for the fractions which contain two ethers. Calculated as percentages on the total phenols the amounts are :—Guaiacol 8.6, creosol 3.0, coerulignol 0.32, higher phenols as pyrogallol dimethyl ether 6.9. The middle fractions were not investigated in detail. That boiling from 215 to 230° formed 13 per cent. of the total phenols and probably contained most of the creosol. It corresponded with the fraction 120–130°/20 mm., the methoxyl value of which was 6.8 so that th[illegible] was 4 per cent. of the

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total phenols. The fraction 230–240° was only 3.5 per cent. and its methoxyl value about 9. Assuming coerulignol to be the only ether present, the amount would be 1.7 per cent. The total quantity of creosol would thus be 7 per cent. and of coerulignol 2.0. A mixture containing these amounts and the quantities of the other two ethers already given, namely, 8.6 and 6.9, would have a methoxyl value of 6.9, corresponding closely with the value 7.0 calculated for the total phenols from the methoxyl values of the different fractions given in Table VI. It was found however that the methoxyl value of the mixed phenols before fractionation was 11.0. This indicates that there is decomposition on distillation and that the residual products contain a high proportion of methoxyl.

The Neutral Oils.

The neutral oils which formed 37 per cent. of the tar distillate were pale brown, had d^{30}_{30}=0.980, n^{30}_{D}=1.4985 and a methoxyl content of 4.4 per cent. On distillation at 680 mm., the boiling point ranged from 120° to 300°, the density at 25° of fractions collected every 10° rose from 0.917 to 0.990 and the refractive index at the same temperature from 1.479 to 1.549; 11 per cent. distilled between 200° and 210° and 32 per cent. between 230° and 260°. The fractions boiling between 240–280° darkened on exposure to air but the others were unchanged. On treating 25 g. with 80 per cent. sulphuric acid, which according to Egloff and Morrell (*Ind. Eng. Chem.*, 1926, **18**, 354) reacts only with unsaturated bodies, a residue of 14.0 g. was left, and on nitration all dissolved except 0.5 g. To determine the phenolic ethers, 20 g. were demethylated with hydrochloric acid and the resulting phenols extracted with sodium hydroxide, 5 g. of phenols and 13 g. of unattacked residue being obtained. Since the methoxyl content of the original oil was 4.4 per cent. it may be calculated that 29.4 per cent. of phenolic ethers with a methoxy-content of 15 per cent. were present. The latter value is low, the figure for creosol methyl ether being 24.6. It is to be inferred, apart from experimental error which may be large, that the phenolic ethers present were of comparatively high molecular weight or that some bodies soluble in sodium hydroxide were produced by hydrolysis from compounds other than methoxy-derivatives. In either case it is improbable that polymethoxy-compounds were present in appreciable quantity. The composition of the neutral oil thus appears to be: unsaturated compounds 44, phenolic ethers 30, other aromatic derivatives 24, paraffins 2 per cent.

Summary.

1. Bhadravati refined tar has been separated by treatment with organic solvents into (*a*) a brittle solid, 13–18 per cent. according to the solvent used; (*b*) neutral oils, 35 per cent; (*c*) phenols and other acidic bodies, 51–44 per cent. The latter on distillation yielded 28–30 per cent. of volatile compounds and a resinous residue.

2. Direct extraction of the tar with sodium hydroxide solution gave only 20 per cent. of neutral oils, but the volatile phenols amounted to 30–35 per cent.

3. On distillation at reduced pressure the average amount of distillate was 35 per cent. and on redistillation 33 per cent. This contained acids 1.66, bases 0.41, phenols 60 and neutral oils 37 per cent.

4. About 25 per cent of the acids consisted of propionic and butyric acids the remainder being a complex mixture of phenolic acids and possibly higher aliphatic acids.

5. The phenols which formed the greater part of the distillate yielded after demethylation 26 per cent. of monophenols and 37 per cent. of polyphenols corresponding with 44 per cent. of original phenolic ethers, the loss on demethylation being considerable. Among the monophenols the following were identified :—Phenol (0.5), *o*- and *p*-cresols (6.3), *m*-cresol (2.4), 1:4:5-, 1:3:5-, 1:2: 4-xylenols and probably the 1:3: 4-isomer (7.8 per cent.). The higher monophenols boiling from 215° to 240° and forming 6.2 per cent. of the total phenols could not be separated sufficiently for identification. The polyphenols were present partly as such and partly as methylated derivatives, but the method adopted did not enable a separate examination of the two classes to be made. The more important methylated products were guaiacol (8.6), creosol (7), coerulignol (2.0), pyrogallol dimethyl ether and higher boiling ethers calculated as this substance (6.9 per cent.). In all cases the figures for the quantities must be regarded as very approximate owing to incomplete separation and the decomposition occurring at every stage.

6. The neutral oils forming 37 per cent. of the distillate were found to contain 44 per cent. of unsaturated compounds, 30 per cent. of phenolic ethers (probably a high value, 20 being more likely), 24 per cent. of other aromatic derivatives and 2 per cent. of paraffins.

II. THE PRODUCTION OF LAMP-BLACK.

A possible method of utilizing the tar or certain fractions of it would be to burn it for the production of lamp-black. Some experiments were consequently made with a view to determining the suitability of the material for this purpose. Small-scale trials showed at once that the incomplete combustion of the tar was difficult to effect. On heating in a crucible to about 140° the vapours could be ignited, and on lowering a water-cooled metallic cup into the flame until the smokiness reached a maximum, a deposit of carbonaceous material was obtained. After a short time, however, a crust formed on the surface of the liquid which had to be broken in order to allow the combustion to proceed regularly and when no more volatile material could be obtained, a large residue remained in the crucible.

Experiments were made with samples of the refined tar used in part I and also with the crude settled tar and a variety described as ' pitch tar '. The average yields were 4.5, 5.5 and 4.0 per cent. respectively, and the products were almost identical in properties, bearing a close resemblance to carbon black of good quality.

The specific gravity of all three samples was 1.70 and the ' free carbon ' after toluene extraction 97 per cent., corresponding figures for the carbon black examined being 1.74 and 94.5. The acetone extract was nearly colourless.

Under the microscope the black was seen to consist of fairly uniform, opaque, amorphous particles approximately the same size as those of the carbon black. On attempting to make a comparison with samples of ' lamp black ' bought locally it was found that these were grossly adulterated, the ash varying from 15 to 63 per cent.

IV. THE CRACKING OF WOOD TAR.

As the quantity of useful constituents of the tar which could be obtained by distillation was comparatively low, it was considered of interest to see if the yield could be increased by cracking. To effect this the tar was run at the rate of about 5g./min. into an iron tube 4 cm. in diameter and 100 cm. long inclined to the horizontal at an angle of 30°, which could be heated electrically. Screw caps were fitted at the ends to enable the tube to be cleaned, an operation which had to be conducted at the end of every experiment as voluminous deposits of pitch were formed which sometimes choked the pipe, particularly in the early experiments in which the tube was less steeply inclined. The products were passed through a condenser and collected in receivers cooled in a freezing mixture. The uncondensed gas was collected in a gas-holder or in some cases partly condensed by cooling to liquid air temperature.

The distillate was separated into acidic bodies and neutral oils by treatment with 20 per cent. sodium hydroxide solution. The gas was analysed for *carbon monoxide, carbon dioxide* and *unsaturated hydrocarbons*, hydrogen and saturated hydrocarbons being determined by difference since they could not be estimated by any simple process in the absence of knowledge regarding the actual gases present. Table I shows the weight of products per 100 g. of tar at 4 different temperatures, the pressure being atmospheric. For comparison the average results of distillation are given in the last line, the maximum temperature being about 260°. Table II gives the composition of the gas.

TABLE I.

Cracking of Tar at Different Temperatures.

Temperature		Weight cracked	Distillate	Gas	Residue	Acidic bodies	Neutral oils
400	..	198	72.2	1.9	25.9	43.4	28.8
500	..	220	64.1	4.8	31.1	43.3	20.8
550	..	160	62.5	6.8	30.7	43.2	19.3
600	..	117	62.4	9.2	28.4	44.0	18.4
Distn.	..	..	36	..	64	22	14

TABLE II.

Composition of the Gases.

Temperature		Carbon monoxide	Carbon dioxide	Unsaturated hydro-carbons	Hydrogen and saturated hydrocarbons
400	..	15.8	14.3	7.9	58.6
500	..	20.8	13.2	11.6	54.4
550	..	34.7	10.6	17.8	36.8
600	..	33.5	4.4	20.1	42.0

From these figures it is evident that cracking gives a far larger yield of volatile products than simple distillation. Variation of temperature over the range studied did not produce as much change in the yield of distillate as might

have been expected, the amount of acidic bodies remaining nearly constant. The neutral oils appeared to decompose progressively with rise in temperature giving pitch and gas containing increasing amounts of carbon monoxide and unsaturated hydrocarbons. The phenolic bodies obtained at 400° and 600° were fractionally distilled and were found to differ somewhat in composition, those obtained at the lower temperature boiling almost entirely between 210° and 270° (87 per cent.) and the others giving 25 per cent. boiling at 190–210° and 45 per cent. at 250–295°. In the absence of larger quantities of material a more detailed examination was not made.

The high yield of volatile products obtained by passing the tar through a hot tube indicates the advisability of examining the method in greater detail and of determining the effect of pressure.

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THE EQUILIBRIUM BETWEEN DIMETHYL ETHER, METHYL ALCOHOL AND WATER.

By N. G. Gajendragad, S. K. Kulkarni Jatkar and H. E. Watson.

In a previous communication (S. K. Kulkarni Jatkar and H. E. Watson, *J. Indian Inst. Sci.*, 1926, **9A**, 71) an account was given of experiments designed to determine the equilibrium constant in the reaction between ethyl ether, ethyl alcohol and water. These were subsequently extended to the corresponding reactions with methyl and *n*-propyl alcohols but the results, although complete 3 years ago, were not published as it was desired to supplement them by theoretical calculations involving the specific heats of the alcohols and ethers which were being measured in these laboratories. It has now been found that specific heat determinations of sufficient accuracy are likely to occupy a longer period than was anticipated and meanwhile the dehydration of methyl alcohol has assumed considerable importance in connection with the synthesis of this compound, so that it seems desirable to place the results on record without further delay.

The equilibrium between dimethyl ether, methyl alcohol and water has received little attention. McKee and Burke (*Ind. Eng. Chem.*, 1923, **15**, 686) in studying the preparation of methyl alcohol from methane deduced that at 300°, 80 per cent. of alcohol would be converted to the ether, a figure which was supported by calculations made by means of Nernst's equation using the available thermal data. Smith and Hawk (*J. Phys. Chem.*, 1928, **32**, 415) have shown that large quantities of methyl ether may be formed during the synthesis of methyl alcohol from carbon monoxide and hydrogen when dehydrating catalysts are used. Brown and Galloway (*Ind. Eng. Chem.*, 1929, **21**, 319 ; 1930, **22**, 175) give 54 per cent. as the proportion of alcohol converted into ether under actual working conditions.

Our experiments indicate that the amount of anhydrous alcohol converted to ether is 88.7 per cent. at 230° and 93.3 per cent. at 130°.

Experimental.

The dehydration of methyl alcohol is somewhat more difficult to investigate experimentally than that of ethyl alcohol owing to the ether being a gas. Preliminary experiments were made using the same apparatus as before and potassium alum as a catalyst. The reaction products were condensed in a tube cooled in liquid air and the gas evolved on warming collected over sodium silicate solution previously saturated with dimethyl ether as recommended by McKee and Burke (*loc. cit.*). Irregular results were obtained at first, the maximum conversion at 230° being 85 per cent. Further experiments indicated an appreciable reduction in the yield of ether at 220°, but between 225° and 240° the effect of temperature was not very marked. Using 99 per cent. alcohol and 100 g. of catalyst in a tube 2.5 cm. in diameter, the amount of unchanged alcohol recovered when the rate of flow was 40 c.c./hour, was nearly twice as much as when the rate was only 20 c.c./hour indicating that the catalyst was far less efficient than in the case of ethyl alcohol, 200 c.c. of which could be

passed hourly in the same apparatus without appreciably diminishing the amount dehydrated.

The necessity of working with small quantities of methyl alcohol adds to the experimental difficulties not only on account of the analytical procedure but also because a small change in temperature of the catalyst may alter the quantity of water it contains and affect the composition of the reaction products. A new type of apparatus was therefore constructed in which the temperature of the catalyst could be maintained constant to less than 2° throughout a length of 60 cm. and the variation during an experiment was not more than 1°. This apparatus was oil-heated and is shown diagrammatically in Fig. I.

The copper tube C containing the catalyst was 110 cm. long and 1.3 cm. in diameter. It was placed inside a wider tube D leaving an annulus of 1.4 mm. into which a spiral copper wire E was inserted to form a baffle. The joints between the tubes were made oil-tight by means of stuffing boxes, and oil could be circulated through the system by a pump H connected to an external pipe system as shown, and driven by a motor J and reduction gearing I. Heat was supplied to the oil electrically by an insulated winding between the pump and the point G and loss of heat from the larger tubes was prevented by two independent auxiliary windings, the whole apparatus being thickly lagged. By suitably adjusting the heating currents, the thermocouples at F and G could be made to indicate substantially the same temperature. The actual temperature was measured by a thermometer at F, which was an open tube to allow the oil to expand.

Alcohol was admitted from the burette and capillary dropper A to the copper spiral B in which it was volatilised and superheated. Before coming into contact with the catalyst it was passed through a layer of pumice 20 cm. long, there being a similar packing of this material at the outlet end of the tube so that the catalyst itself was entirely within the region of uniform temperature. McKee and Burke's method of measuring the gas over sodium silicate solution was found unreliable and consequently the reaction products were passed into the vessel K cooled in a freezing mixture and the uncondensed gas after traversing the weighed calcium chloride tube L was absorbed in concentrated sulphuric acid in the vessel M.

Analysis of the Reaction Products.

Preliminary experiments showed that calcium chloride absorbed both water and methyl alcohol vapours but not dimethyl ether. At the conclusion of each experiment the tube K was warmed to about 60° to drive the dissolved gas into the absorption bulb M. Nearly all this gas was liberated below 0° so that the quantity of methyl alcohol and water vapour carried with it was small and the increase in weight of the calcium chloride tube L was rarely more than 1 per cent. of the total distillate. It was not necessary, therefore, to determine accurately the relative amounts of the two substances. No data are available for the composition of the vapour of water-methyl alcohol mixtures at low temperatures and hence it was assumed that the partial pressure of each constituent was a linear function of the molecular percentage in the liquid. The ratio of the quantities of the two substances in the vapour phase when so calculated does not vary greatly with temperature and hence 0° was assumed as an average.

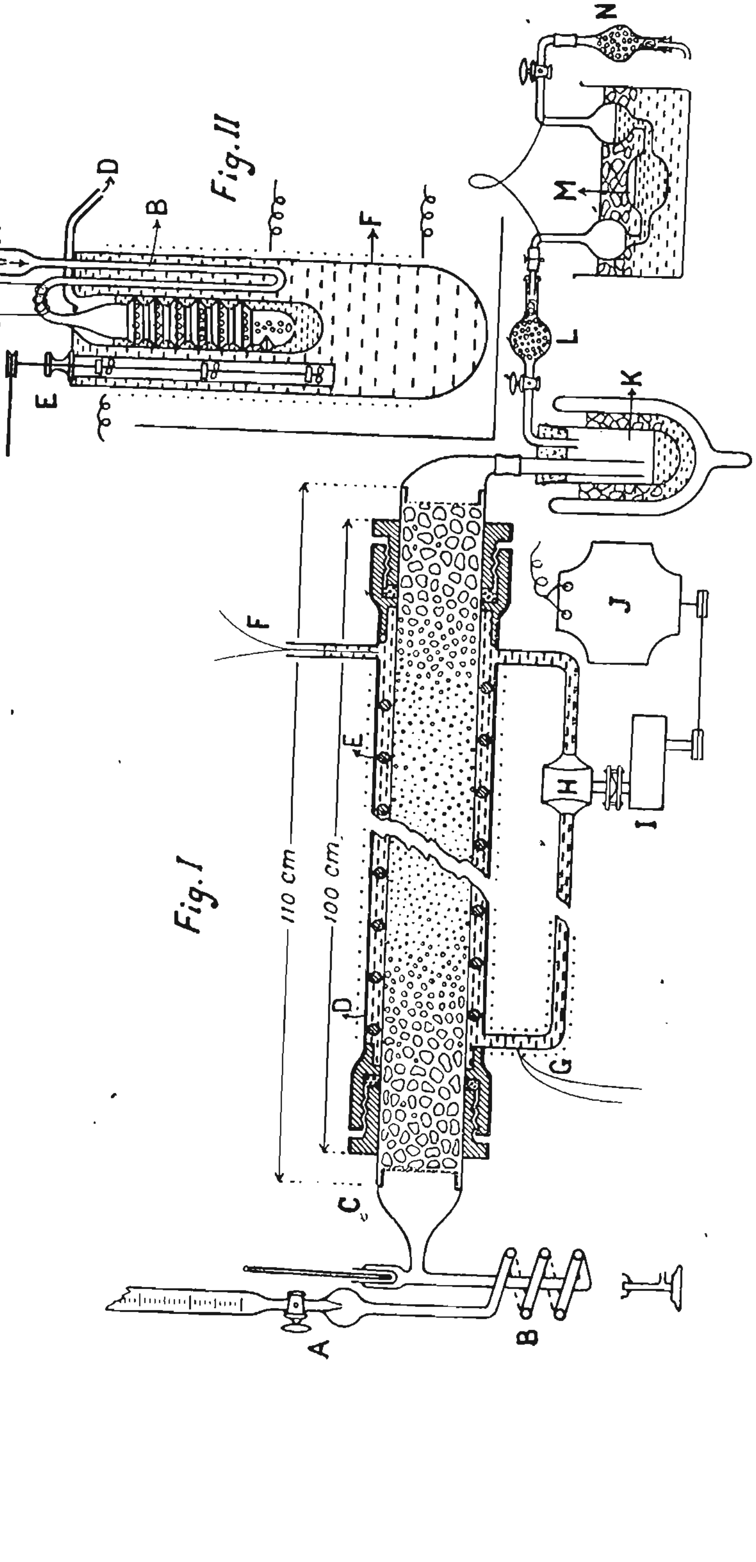
Fig. I
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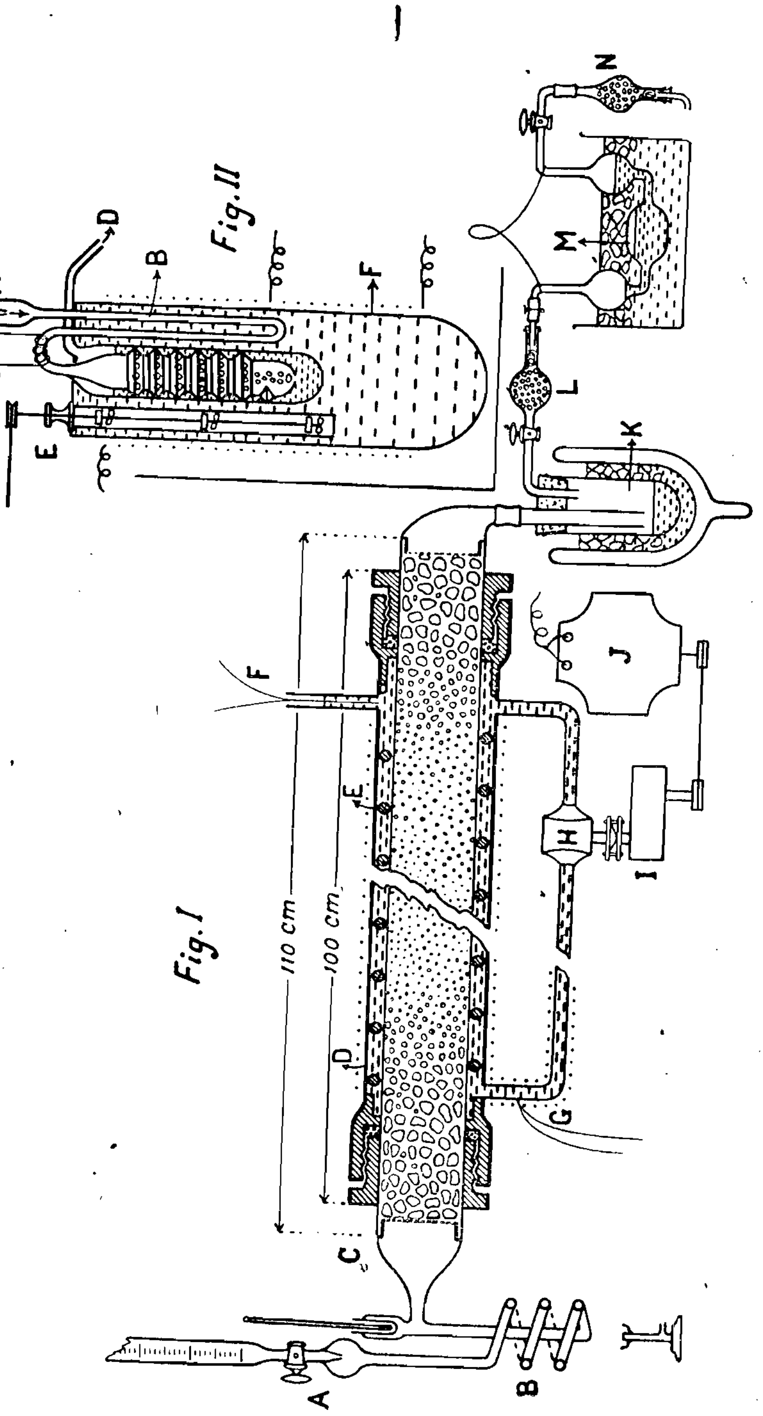


Fig. I

Fig. II

The following is a typical experiment to illustrate the method of calculation :—

Expt. 190. Increase in weight of tube K	.. ..	2.57 g.
,, ,, tube L	.. ..	0.05 g.
,, ,, bulb M	.. ..	5.49 g.
Total	.. ..	8.11 g.

Density of liquid in K at 15° :—0.9486.
Percentage of methyl alcohol :—33.2 by weight, or 21.8 mols. per cent.
Vapour pressures at 0° :—methyl alcohol 29.6, water 4.6 mm.
Vapour pressure of alcohol in mixture :—6.5 mm. ; of water 3.7 mm.
Weight of alcohol in L :—0.04 g. ; weight of water, 0.01 g.
Total : ether, 5.49 ; alcohol, 0.90 ; water, 1.72 g.
or 0.119 0.028 0.095 mols.
Hence K, the equilibrium constant $=119\times95\div28^2=14.4$.

The term 'percentage conversion' which affords a better physical conception of the extent of the reaction than the equilibrium constant K, is, strictly speaking, only applicable to the case of anhydrous alcohol, whereas K can always be calculated whatever the composition of the initial mixture. When anhydrous alcohol is used, every value of K corresponds with a definite conversion ; for instance, in the example just given K would have the value 14.4 if 88.5 per cent. of anhydrous alcohol were decomposed on passing through the catalyst. It is convenient when using dilute alcohol or mixtures to retain the term 'percentage conversion' bearing in mind that it does not refer to the actual liquids employed but to the conversion of anhydrous alcohol which would yield the same value of K. This has been done in the following tables.

Results.

With the new apparatus just described results were more regular than previously, but, at first, by no means satisfactory. In 20 experiments with 99 per cent. alcohol at 230°, conversions from 66 to 84 per cent. were obtained under identical conditions and when the rate of flow was reduced, even lower values were obtained. With fresh catalyst higher mean values were found, but irregularities were still apparent as may be seen from some typical results shown in table I. All rates are expressed in c.c. per hour and the experiments are numbered in the order in which they were performed.

TABLE I.

Formation of Methyl Ether from Methyl Alcohol.

Expt. No.	Rate	Conversion	Expt. No.	Rate	Conversion	Expt. No.	Rate	Conversion
52	26	77	63	15	44	81	8	74*
53	12	83	64	10	62	82	8	75*
54	24	79	65	4	64	83	15	66*
55	33	70	61	3.5	69	84	9	68*
56	18	78	67	3	74	85	8.5	67*
57	14	78	71	11	42	90	5.5	76
58	8	83	72	11	48	91	6	73

* 220°

The first group of experiments (52-58) was made with 98 per cent. alcohol at 230°, and experiments 90, 91 with similar conditions indicate a decrease in activity of the catalyst. The second group (63-72) shows the effect of using 85 per cent. alcohol; the rate of conversion is very low and the last two experiments after 4 runs with 96 per cent. alcohol give even smaller values. Experiments 81-85 were made at 220° and the difference is not very marked.

It thus appeared that there was a tendency for the catalyst to become inactive and this was confirmed by occasionally passing ethyl alcohol, which reacts much more easily than methyl alcohol and comparing the results at different times. It also seemed likely that irregular and low values were due to channelling of the catalyst owing to the vibration of the circulating pump; the whole apparatus was consequently tilted at an angle of 45° and this gave greatly improved results. Table II shows a series of values with a fresh batch of catalyst using 98.7 per cent. alcohol at 230° in the order in which they were determined.

TABLE II.

Rate	9.6	9.6	9.6	3.2	3.2	9.6	12.8	19.2	9.6
Conversion ..	86.8	88.5	89.0	88.2	87.5	87.0	86.3	76.0	85.1

In these experiments 6 to 12 grams of distillate were collected for analysis and to ensure equilibrium, alcohol was passed over the heated catalyst for at least half an hour before the absorption tubes were attached. When the last experiment was made, the catalyst had been used for about 30 hours and the somewhat lower result may have been due to slight deterioration.

As these results appeared sufficiently concordant, a further series of experiments was made to determine more accurately the most favourable temperature for the reaction, preliminary experiments having indicated 225-230°. Table III shows the results in the order in which they were obtained, each value being the mean of two agreeing within 1 per cent. using 98 per cent. alcohol at the rate of 10 c.c. an hour.

TABLE III.

Temperature	Conversion	Temperature	Conversion
160	0	230	79.9
170	9.8	240	83.3
180	23.3	250	85.5
190	35.5	230	73.0
200	50.5	210	52.0
210	62.9	250	84.0
220	69.5		

There was very little reaction below 170° but above this temperature a steady rise was found in the conversion, that is to say, in the reaction velocity. At the higher temperatures, equilibrium was approached and consequently the change in the conversion for equal increments of temperature became less, but it was evident that the greatest velocity reached was at 250°, the highest temperature tried. This was not however a practicable working temperature owing to its causing a loss in activity of the catalyst as shown by the fact that the conversions at 230° and 210° after heating were decidedly less than before. 230° was therefore adopted as the most suitable temperature in subsequent

experiments, the rate of conversion being sufficiently rapid and the deterioration of the catalyst not excessive.

The Reverse Reaction.

In order to obtain more definite information regarding the equilibrium point it was desirable to approach it from both directions. Previous experience with ethyl alcohol indicated that the reverse reaction would probably take place very slowly and this was found to be the case, reliable results being difficult to obtain.

Methyl ether was generated from its solution in sulphuric acid by adding water at a regulated rate, washed with very dilute alkali and passed through a humidifier consisting of a copper tube packed with moistened Lessing rings maintained at about 86°. Spray was removed, the gases passed into the apparatus and the product analysed as before.

The first experiments were irregular and indicated the formation of only 4 to 6 per cent. of methyl alcohol. A fresh batch of catalyst was prepared with which more satisfactory results were obtained. These are shown in table IV. The rate recorded is the number of grams of reaction products collected per hour and the temperature 230°.

TABLE IV.

Expt. No.	Mols. $Me_2O : H_2O$	Rate	K	Conv.	Expt. No.	Initial mixture	Rate	K	Conv.
185	41 : 59	8	15.7	88.8	187	99% MeOH	7.8	15.1	88.6
186	60 : 40	4	16.4	89.0	188	,,	7.8	15.2	88.6
189	48 : 52	7	17.4	89.3	193	,,	7.8	14.9	88.5
190	55 : 45	6	14.4	88.5	194	,,	7.8	13.9	88.2
191	60 : 40	4.5	19.8	89.9	195	,,	4.0	16.0	88.9
192	65 : 35	2	20.0	90.0					

The experiments were conducted in the order in which they are numbered and it will be noted that although the values of K obtained for the direct reaction experiments 193 and 194 are slightly lower than in 187 and 188, the difference when expressed as percentage conversion is very small and within the limit of experimental error so that any loss in activity of the catalyst was slight. The reverse reaction experiments 185, 186 and 189 gave, as might be expected, slightly higher values for K indicating that equilibrium was not quite reached, but they suffice to restrict the true point of equilibrium within very narrow limits. The high values in experiments 191, 192 and 195 and the low one in 190 are no doubt due to experimental error, considerable difficulty being experienced in keeping conditions steady during the two to four hours necessary to accumulate sufficient material for analysis in the experiments at very low rates.

A few attempts were made to obtain the value of K using a mixture of methyl alcohol and water in the ratio 65 mols. of alcohol to 35 mols. of water, but the reaction was so slow that equilibrium could not be attained. With rates of flow of 9.5 and 8.0 g. per hour, the values were 5.1 and 8.2 corresponding with conversions of 81.9 and 85.1 per cent. respectively. When the rate was diminished to 2.5 g. per hour, K rose only to 11.6, or 87.2 per cent. conversion. This reduction in reaction velocity is analogous to that previously observed with ethyl alcohol.

The mean result of all these experiments is that at 230° the equilibrium point for anhydrous methyl alcohol is reached when 88.7 per cent. has decomposed, the corresponding value of K being 15.4.

The Reaction at 130°.

The figures given in table III, make it evident that there would be little chance of determining the equilibrium point of the reaction at temperatures much removed from 230° if an alum catalyst were used. A few attempts at 200° confirmed this supposition, values of 3.6 and 225 being obtained for K in the direct and reverse reactions respectively.

As it was desirable to study the reaction at two temperatures at least, it was decided to experiment with sulphuric acid as catalyst, either alone, or mixed with aluminium sulphate as recommended by Senderens (*Compt. rend.*, 1910, **151**, 392). The reaction vessel used is shown in Fig. II and consisted of a Friedrich's spiral wash bottle 4 cm. in diameter completely immersed in an electrically heated oil thermostat with a powerful stirrer. The temperature was hand-controlled and usually did not vary by more than 0.5° during an experiment. The reaction mixture was admitted at A at a controlled rate and was volatilised and superheated to the bath temperature in the tubes B and C. The products issued at D and were examined in the same way as before.

A disadvantage of sulphuric acid catalysts was their low activity which made it necessary to employ 100 g. in order to collect the reaction products at the rate of about 10 c.c. an hour. Analysis showed that the catalyst when equilibrium had been established was not sulphuric acid or a hydrate, but a mixture with the approximate composition $H_2SO_4.4H_2O$, 0.25MeOH at 128° and $H_2SO_4.2.6H_2O$, 0.20MeOH at 140° and thus if the temperature varied or the gas was passed too rapidly for equilibrium to be attained, the catalyst might change its composition, giving out or absorbing methyl alcohol and water. It was not convenient to collect more than about 15 c.c. of reaction products, only one-tenth of which or less was methyl alcohol and consequently it was very easy for large errors to be introduced.

In order to compensate the effects produced by these changes the emerging mixture was analysed completely when possible and was assumed to be in equilibrium. For slow rates this was probably true to a first approximation, the substances introduced reacting not only as they would with a solid catalyst but also combining with what may be regarded as the surplus products of the catalyst at the moment. It follows that the mixture issuing from the catalyst need not necessarily correspond in amount or in composition with the reactants introduced. The weight of alcohol and water evolved on raising the temperature of the catalyst 0.5° was approximately 1 g. but the difference between the weight introduced and that obtained frequently exceeded this amount in spite of an apparent constancy in temperature. The reason for this was not apparent, but when considering the results, those experiments for which the discrepancy was large were given less weight. Calculations of the equilibrium constant made with the assumption that the change in weight was due to absorption or evolution of water and a small proportion of methyl alcohol by the catalyst gave more concordant values for K in many cases but the composition of the distillate so calculated did not agree with the analytical values.

Some of the preliminary results are shown in table V. The equilibrium constant and hence the percentage conversion has been calculated by assuming that the amount of water in the distillate was equivalent to the dimethyl ether obtained, the balance being alcohol. The values are in consequence by no means accurate. In a few cases (indicated by a star) the liquid distillate was analysed. Some of the results refer to two or more experiments with similar rates of flow, the products of which were mixed before examination.

TABLE V.

99.5 per cent. Methyl Alcohol with Sulphuric Acid and 5 per cent. Aluminium Sulphate.

No.	Temp.	Rate g./hr.	MeOH added g.	Total distillate g.	Me_2O	Conversion per cent.
11	125	18	41.5	45.85	25.7	83.0*
12	125	13	26.95	27.95	17.25	86.7
13	125	9	18.7	18.3	11.6	88.1*
14	125	8	7.8	8.33	5.36	90.1
18	125	14	21.1	19.77	12.70	88.9
19	125	14	20.2	19.88	11.83	83.3
20	125	8	15.7	13.80	9.16	93.1
21	126	7	18.7	20.24	13.73	89.6*
22/3	127	9	17.9	16.84	10.64	88.5*
24/5	128	5.5	18.3	18.25	11.75	89.1*
26/7	128	17	18.5	19.43	10.46	75.8

After conducting some experiments on the reverse reaction additional determinations were made at a slightly higher temperature and, the reaction-products examined in every case. These results are shown in table VI.

TABLE VI.

99.5 per cent. Methyl Alcohol, Sulphuric Acid.

No.	Temp.	Rate g./hr.	MeOH added g.	Total distillate g.	Me_2O	H_2O	MeOH	Conversion per cent.
41/3	129	12.5	24.9	22.97	15.56	5.13	2.28	89.6
44/5	128	17	17.2	16.00	9.91	3.95	2.14	86.7
46/7	129	4.5	17.0	16.01	10.85	3.88	1.28	92.2
48	129	8	12.1	10.73	7.08	2.65	1.00	90.7
49	128*	4.5	11.6	13.23	6.38	5.67	1.20	92.1
50	140	3.5	17.3	16.90	11.10	4.46	1.34	92.3
51/2	130	12	25.1	26.14	16.35	6.77	3.02	88.6
54/5	130	7	25.8	24.56	15.89	6.35	2.32	90.6
62/3	130	7.5	16.5	18.44	11.87	4.66	1.91	89.6
64	130	12.5	14.5	13.76	8.74	3.51	1.48	89.3
65	130	4.5	18.3	17.63	12.08	4.11	1.44	91.9
66	130	5.5	12.4	12.49	8.25	3.17	1.07	91.6

* Irregular.

Newly made catalysts required a considerable time to attain equilibrium. The one used for expts. 41-49 consisted of 75 g. concentrated sulphuric acid and 5 g. aluminium sulphate to which was added 62 g. methyl alcohol, the whole

being heated until no more gas was evolved. When these experiments were completed, a fresh catalyst was prepared in a similar manner and heated to 150°. On passing methyl alcohol, the product separated into 2 layers owing to the formation of methyl sulphate. At 140° the side reaction was less, but traces of methyl sulphate were found and after a few experiments the catalyst appeared to decrease in activity. Expt. 50 was made at this temperature but subsequent determinations were restricted to 130°.

Expts. 51-59 were made with the same type of catalyst but after No. 59 which was made by passing methyl ether and water to investigate the reverse reaction, low results were obtained on again passing methyl alcohol. A new catalyst was then made consisting of 62 g. sulphuric acid and 38 g. water through which methyl alcohol vapour was passed for several hours. This gave satisfactory results and was used for the remaining experiments. The values for the conversion have been plotted against the rate of flow in Fig. III, and it will be seen that even at the slowest rates measured, equilibrium is not attained.

The extrapolation to zero rate is rather wide and consequently experiments were made upon the reverse reaction. Water was added from the burette and a steady stream of dimethyl ether generated by dropping water into a solution of the gas in sulphuric acid, admitted through a side tube. The rate was measured approximately by counting the bubbles. The results are given in table VII which shows the initial and final weights of the different constituents. The initial weights of dimethyl ether were obtained by calculation and are not accurate.

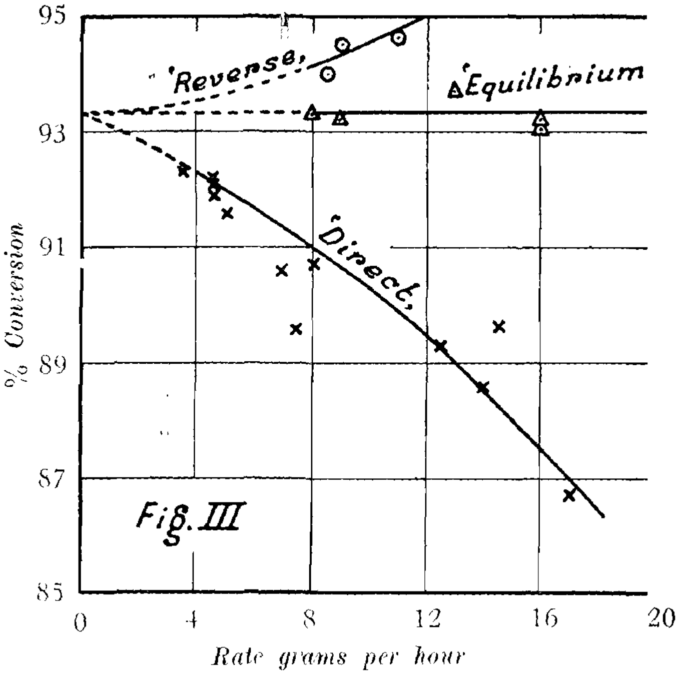
TABLE VII.

Reverse Reaction and Miscellaneous Mixtures.

			Initial			Final			
No.	Temp.	Rate g./hr.	Me_2O	H_2O	MeOH	Me_2O	H_2O	MeOH	Conversion
59	130	8.5	10.0	63.0	0	9.25	7.04	1.06	94.0
67	130	11	11.8	6.9	0	11.10	6.46	1.07	94.6
68	130	9	16.6	7.3	0	15.58	8.47	1.50	94.5
69	130	5	13.1	0	0	12.48	4.30	0.89	94.8
56	130	16	11.0	5.6	1.9	11.44	4.79	1.20	93.2
57	130	13	10.2	4.6	1.5	10.53	4.60	1.04	93.7
58	130	8	10.9	4.65	1.55	11.16	5.22	1.23	93.3
70	130	9	11.7	3.25	1.75	12.11	4.23	1.16	93.2
71	130	16	9.1	4.55	2.45	10.07	4.33	1.10	93.1

In expt. 69 no water was added and it will be seen that the results are not greatly affected, the catalyst temporarily supplying the necessary water.

Expts. 56-58 were made by passing dimethyl ether and a 25 per cent. aqueous solution of methyl alcohol, the mixture approximating to the equilibrium mixture. This causes a minimum amount of alteration in the composition of the catalyst at the point of entry and results in a longer periods of effective contact. Expts. 70 and 71 were of a similar nature but with a 35 per cent. solution of alcohol.



Extrapolation of the results in table VI shown in Fig. III indicates a value for the conversion at zero rate between 93 and 94 per cent. and the reverse reaction shows that the figure is certainly less than 94.5. In view of the slow rate of this reaction the mean value is probably at least 1 per cent. too high. The figure 93.3 would agree well with the results of these determinations and is in accordance with the results for the "equilibrium mixture". It may therefore be taken as the most probable value. The corresponding value of the equilibrium constant is 48.5, but since the values of K for 93 and 94 per cent. conversions are 44.1 and 61.5 respectively, it is evident that this constant is not known with any great degree of accuracy.

If the above result be taken in conjunction with the value $K_1=15.4$ at 230°, the heat of reaction calculated according to the equation

$$Q=4.58 \log K_2/K_1 \, . \, T_1T_2/(T_1-T_2)$$

is 4.62 Cals.

Taking Thomsen's data for the heats of combustion of methyl alcohol (182.2 Cals.) and dimethyl ether (349.4 Cals.) and 10.6 Cals. as the molecular latent heat of vaporisation of water at 18° the heat of reaction is 4.4 Cals. in fair agreement with the value calculated above.

Summary.

1. The equilibrium between dimethyl ether, methyl alcohol and water has been investigated by a continuous flow method using an alum catalyst at 230° and sulphuric acid at 130° the equilibrium point being approached from both directions.

2. The value of the equilibrium constant was found to be 15.4 at 230° and 48.5 at 130° corresponding respectively with the conversion of 88.7 per cent. and 93.3 per cent. of anhydrous alcohol into ether and water.

3. The heat of reaction calculated from the equilibrium constants agrees as closely as can be expected with the value from thermal data.

4. With the alum catalyst the reaction is much less rapid than in the case of ethyl alcohol. The velocity increases with rise in temperature up to about 250° but at this temperature the catalyst loses its activity.

5. Sulphuric acid catalysts cannot be used at temperatures above 130° even with addition of aluminium sulphate owing to the formation of dimethyl sulphate.

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I

I.—ESSENTIAL OIL FROM LEAVES OF *LITSAE ZEYLANICA*, LINN.

By B. Sanjiva Rao.

Litsæ Zeylanica, Linn., belongs to a large genus of about fifty-six species, of which several are indigenous to the Western ghats and other parts of Southern India, growing up to 7,000 feet in the Nilgiris and also found in Bhutan, the Khasia mountains, Java and Sumatra. It is a moderate-sized, ever-green tree whose wood is employed for furniture and house-building, as it is rarely attacked by insects and will last many years even in the ground and exposed to weather. The leaves and bark resemble those of the closely allied cinnamon species and are often used for the adulteration of the latter.

The leaves were distilled by Sastry (*Perf. and Essent. Oil Rec.*, 1923, **14**, 271) who obtained 0.6, 0.22 and 0.05 per cent. of oil from fresh, partly dried and dried leaves respectively. The constants for these oils, according to Bennett, have been given in Table I.

The leaves used for the present investigation were obtained in March and November and immediately distilled. The constants of the oils do not indicate any pronounced difference in composition due to seasonal variation. The slightly lower yield in November is probably due to the longer interval between collection and distillation.

A detailed examination of the oil has shown it to be mainly a mixture of terpenes (ocimene and α-pinene) with sesquiterpenes.

The sesquiterpenes are entirely bicyclic and belong to the cadalin group. A crystalline sesquiterpene dihydrochloride (m.p. 100-101°) was obtained, apparently a derivative of a hydrocarbon hitherto unknown, as no hydrochloride having this melting point is recorded, the only sesquiterpene dihydrochlorides melting in this region being that of (1) *dl*-cadinene (m.p. 105-106°) and (2) the tricyclic sesquiterpene heerabolene (m.p. 99-100°) isolated by Friedrichs (*Arch. Pharm.*, 1907, **245**, 208). Even if the dihydrochloride is identical with the latter, it appears certain that there is no tricyclic sesquiterpene resembling heerabolene in this oil, and it is intended to examine the compound further when a fresh supply of material becomes available.

One of the sesquiterpene fractions gave a small yield of a nitrosochloride (m. p. 165°) indicating α-caryophyllene. The quantity of sesquiterpene alcohols being small, they were not further investigated. The percentage composition of the oil is approximately as follows:— α-Pinene, 3.5; ocimene, 60.0; terpene alcohols, 1.0; bicyclic sesquiterpenes, 27.0; sesquiterpene alcohols, 3.0.

Experimental.

Two consignments of leaves and terminal branchlets were obtained, one in March and the other in the November following. The first was distilled in

steam on the 5th day after collection and the second after 15 days. The distillates were pale lemon, very mobile oils, and when dried with magnesium sulphate had the constants in Table I which includes for comparison the values found by Bennett.

TABLE I.

Weight of leaves	Yield of oil per cent.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Acid value	Sapn. value	Sapn. value after acetylation
150 lbs.	0·45	0·8376	1·4874	+0·6°	0·4	0·7	41·7
335 ,,	0·39	0·8383	1·4874	+2·0	0·3	0·6	45·6
		0·890	1·4980	+1·0	0·47	22·6	81·6

Phenols and carbonyl compounds were not present in any appreciable quantity.

Distillation of the Oil.—The oil (200 g.) was washed with sodium carbonate to remove free acids and distilled at 100 mm. with a 4-pear Young's column, when the following fractions were obtained:—

TABLE II.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in gms.	Yield per cent. on original oil
1	105—110°	0·8041	1·4803	—2·2°	15·5	7·7
2	110—115	0·7995	1·4817	—1·1	83·0	41.5
3	115—120	0·8013	1·4832	—0·7	24·5	12·3
4	120—125	0·8123	1·4850	+0·2	11·0	5·5
5	130—165	0·8557	1·4902	+0·3	7·0	3·5
6	165—187	0·9111	1·4992	+1·4	49·0	24·5
	Residue				6·0	3·0

The first three fractions which contained most of the terpenes were repeatedly distilled with a 5-bulb Le-Bel column at 100 mm. and the results summarised in Table III.

TABLE III.

Terpenes.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in gms.	Yield per cent. on original oil
A	103—105°	0·8459	1·4660	—10·2°	2·5	1·3
B	105—106	0·8281	1·4723	— 8·4	5·0	2·5
C	106—107	0·8187	1·4732	— 7·6	5·0	2·5
D	107—109	0·8132	1·4760	— 7·6	3·5	1·8
E	109—112	0·8085	1·4792	— 3·4	5·5	2·8
F	112	0·8021	1·4820	— 1·8	6·5	3·3
G	112	0·7980	1·4822	— 1·4	18·5	9·3
H	112—113	0·7960	1·4828	0·0	83·2	41·6

Fractions A, B and C (α-pinene).—Fractions B and C were mixed and fractionated over sodium with a 4-pear column at 7 mm. pressure.

	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$
1	48—50°	0·8384	1·4692	—8·8°
2	50—53	0·8110	1·4772	—4·8

Fractions A and 1 readily gave a nitrosochloride which on purification melted with decomposition at 109°. Fraction 2 gave a much smaller quantity of the nitrosochloride melting at 105-107°.

Fractions F, G and H (ocimene).—The physical properties of these fractions agreed fairly closely with those of the aliphatic terpene ocimene, *viz.*, b.p., 110°/100 mm., d^{30}_{30} 0·7933, n^{30}_{D} 1·4809.

Dihydro-ocimene.—Fraction H (20 c.c.) was dissolved in absolute alcohol (150 c.c.) and sodium (20 g.) gradually added, the mixture being finally heated on a boiling water bath. The reduced terpene after distillation over sodium had the following constants:—B.p., 71-72°/30 mm., d^{30}_{30} 0·7711, n^{30}_{D} 1·4458, $[R_L]_D$ 47·61 (calc. 47·45). Dihydromyrcene prepared by the reduction of myrcene, which is identical with dihydro-ocimene (*Ber.*, 1911, **44**, 2010) was found to have b.p., 70°/30 mm., d^{30}_{30} 0·7712, n^{30}_{D} 1·4465.

Dihydromyrcene tetrabromide.—The hydrocarbon (2·7 g.) from the above experiment was dissolved in a mixture of amyl alcohol (4 c.c.) and ether (8 c.c.), when bromine (6·3 g.) dissolved in amyl alcohol-ether mixture was gradually added to the well-cooled solution. There was no immediate precipitate, but after a few hours in the ice-chest a copious crop of crystals separated, which on recrystallisation from methyl alcohol melted at 87—88°, not depressed by admixture with dihydromyrcene tetrabromide.

allo-*Ocimene.*—Fraction H (20 c.c.) was boiled at 685 mm. pressure for 15 minutes and on distillation had the following constants, showing partial conversion into *allo*-ocimene: d^{30}_{30} 0·8092, n^{30}_{D} 1·5054.

Terpene alcohols.—Fraction 4 and the residue remaining after the distillation of the terpenes (Table III) were mixed and saponified with alcoholic potash. The distilled product (3 g.) gave the following constants:—B.p., 88-95°/6 mm., d^{30}_{30} 0·8578, n^{30}_{D} 1·4892, $[\alpha]^{30}_{D}$ —1·2°. No derivatives could be obtained.

Sesquiterpenes.—Fractions 4, 5 and 6 were fractionated and the product boiling at 105-135°/7 mm. was distilled under 8 mm. pressure over sodium giving the following fractions:—

TABLE IV.

Sesquiterpenes.

Fraction	B.P.	d^{30}_{30}	n^{30}_{D}	$[\alpha]^{30}_{D}$	$[R_L]_D$	Weight in gms.	Yield per cent. on original oil
a	108—110°	0·8969	1·4956	—3·9°	66·45	4·0	2·0
b	110—120					0·5	0·2
c	121—123	0·9012	1·4986	+1·0	66·43	33·0	16·5
d	123—125	0·9055	1·5011	+1·3	66·37	13·5	6·7
e	125—126	0·9107	1·5034	+3·1	66·22	6·5	3·2

Determination of sesquiterpenes.—Fractions *c* and *d* were mixed and fractionated into 8 fractions of which 1 and 8 had the constants given below:—

	B.P./11 mm.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	$[R_L]_D$
1	120—123°	0·8966	1·4956	—3·9°	66·46
8	128	0·9118	1·5050	+3·2	66·36

As the figures for molecular refraction of bicyclic and tricyclic sesquiterpenes are 66·15 and 64·45 respectively, the values obtained leave no doubt as to there being a mixture of only bicyclic sesquiterpenes.

Sesquiterpene dihydrochloride.—A solid hydrochloride (0·6 g.) was readily obtained when an acetic acid solution of fraction *c* (8 g.) was treated with hydrogen chloride: it crystallised from ethyl acetate in plates m.p. 100–101° (*Found*: Cl, 24·9; $C_{15}H_{26}Cl_2$ requires Cl, 25·6 per cent.), and was optically inactive. It may be identical with the hydrochloride obtained from *dl*-cadinene (m.p. 105–106°), or from heerabolene, which melts at 99–100° according to Friedrichs (*loc. cit.*). Heerabolene is a tricyclic sesquiterpene giving a dihydrochloride just as tricyclic copaene gives bicyclic cadinene dihydrochloride.

Fraction a (α-caryophyllene).—On treatment with amyl nitrite and hydrochloric acid, a nitrosochloride was obtained which on recrystallisation from alcohol melted at 165°. Attempts to prepare the caryophyllene alcohol were not successful.

Dehydrogenation with selenium (cadalin).—Fractions *c*, *d*, *e* were each separately heated with selenium powder at 260° during 24 hours. The product obtained by distillation over sodium gave in all cases excellent yields of cadalin picrate, m.p. 115° (mixed melting point 115°), showing that the sesquiterpenes belong to the cadalin group.

Sesquiterpene alcohols.—The fraction boiling above 135°/6 mm. had the following constants:—B. p. 135–145°/6 mm., d_{30}^{30} 0·9376, n_D^{30} 1·5026, $[\alpha]_D^{30}$+4·8°. A small quantity treated with sodium reacted vigorously, but the quantity of product was too small for further investigation.

Acids.—The sodium carbonate washings and the alkali liquor from saponification of esters were together treated with sulphuric acid and the volatile acids distilled in a current of steam. The silver salt was prepared after neutralisation with ammonia solution (Found: Ag, 51·1; silver *iso*valerate requires Ag, 51·7 per cent.).

II.—ESSENTIAL OIL FROM FLOWER-HEADS AND STALKS OF *ANDROPOGON KUNTZEANUS*, HACK, *VAR. FOVEOLATA*, HACK.

By B. Sanjiva Rao.

For the purpose of examining the oil from *Andropogon odoratus*, Dna, Lisboa, a quantity of grass, believed to be of this species was collected at Lonawala near Poona. A specimen was, however, identified by R. N. Parker, the Forest Botanist at Dehra Dun as *Andropogon Kuntzeanus*, Hack, *var. foveolata* Hack, an opinion confirmed by the botanist at Coimbatore who stated that *Andropogon odoratus*, Dna, closely resembles this species, but is aromatic. Hooker (*Flora of British India*, 1897, **7**, 177) dealing with *A. odoratus*, Dna, says 'The compressed sheaths, panicled spikes, villous gl. 1 of the sessile spikelets, and aroma, seem to distinguish this from *A. Kuntzeanus*, which it strongly resembles in a dry state.' Thus, although the two grasses are similar, the differences are sufficient to admit of definite discrimination and the identity of the specimen collected in the present case may be regarded as established. The lack of aroma indicated the absence of an admixture of *A. odoratus*. On steam distillation the grass yielded 0·27 per cent. of oil, a quantity greater than was expected. About 40 per cent. of this oil consisted of bicyclic tertiary sesquiterpene alcohols, belonging to the cadalin group. About 10 per cent. of solid *l*–camphene was isolated with an equal amount of material giving a good yield of the chief derivatives of *l*–camphene, but this could not be solidified. Probably there is present an isomeric form of camphene similar to that found in citronella oil (Gildemeister and Hoffmann, *The Volatile Oils*, 1916, **2**, 225). Tricyclene could not be detected, but α-pinene and borneol were present in small quantities.

Experimental.

The grass was cut 2–3 inches above ground and included inflorescence and stalk (moisture 33·3 per cent.): 252 lbs. yielded 203 g. of oil, or 0·27 per cent. calculated on the dry grass, on distillation in steam. This oil was deep brown and had a characteristic sesquiterpenic odour. The more important constants were:—d_{30}^{30} 0·9208; n_D^{30} 1·4813; $[\alpha]_D^{30}$ — 36·5°: acid value 1·7; ester value 0·3; ester value after acetylation 80·8. There was no appreciable absorption either with sodium sulphite or potassium hydroxide. Gildemeister and Hoffmann (*loc. cit.*, 247) give for *A. odoratus* d_{31}^{31} 0·931; $[\alpha]_D$ — 22·75°.

The oil (165 g.) was saponified with alcoholic potash, washed free of alkali and dried over anhydrous magnesium sulphate. The saponified oil (150 g.) distilled under diminished pressure (100 mm.) using a Young's column yielded the following fractions:—

TABLE I.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in grams	Yield per cent. on saponified oil
1	90— 95°	0·8626	1·4653	—77·2°	22·9	15·3
2	95—105	0·8636	1·4663	—77·8	18·0	12·0
3	105—110	0·8670	1·4676	—74·3	9·0	6·0
4	110—115	..	1·4685	..	3·2	2·1
5	120—140	..	1·4708	—56·6	5·8	3·9
6	140—150	0·9073	1·4800	—28·2	18·0	12·0
7	175—200	0·9378	1·4887	— 3·8	27·0	18·0
8	200—215	0·9670	1·4995	— 4·6	37·0	24·7
	Residue and loss				9·1	6·0

The first three fractions contained the greater part of the terpenes and were redistilled repeatedly under 100 mm. pressure, the following fractions being obtained:—

TABLE II.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in grams	Yield per cent. on saponified oil
a	90— 91°	0·862	1·4643	—73·3°	3·0	2·0
b	91— 93	0·861	1·4654	—79·3	19·8	13·2
c	93— 94	0·861	1·4658	—77·1	5·8	3·9
d	94— 96	0·862	1·4663	—76·4	5·2	3·5
e	96—100	0·861	1·4668	—74·3	3·6	2·4
f	100—110	..	1·4678	..	1·5	1·0
g	110—118	..	1·4693	—56·2	1·5	1·0

Fraction a.—On treating this fraction (1 g.) with amyl nitrite and hydrochloric acid a small quantity of solid nitrosochloride was obtained, but the amount was insufficient for satisfactory purification. It is probable that a small proportion of α-pinene, which boils at this temperature and gives a nitrosochloride, is present.

Fractions b, c, d *and* e.—Each of these fractions on keeping in the ice-chest partly solidified. The solid was separated and the combined liquid portions were distilled from sodium and then fractionated under 14 mm. pressure with the following results:—

B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Yield per cent. on saponified oil	$[R_L]_D$ Found	$[R_L]_D$ Calc. for camphene
55°	0·8592	1·4657	—70·4°	14·0	43·80	43·91
55	0·8602	1·4661	—70·5	4·0	43·82	..
55—57	0·8591	1·4661	—74·2	3·0	..	..

A further quantity of solid camphene was obtained by freezing these distillates. The process of distillation and freezing was repeated several times until the final fraction (12 g.) remained liquid. The *l*-camphene (14 g.; 9.3 per cent. on the whole oil) was isolated, had m.p. 45° and $[\alpha]_D^{30}$ —83.2° in absolute alcohol (*C*= 8·180); *it was converted into isoborneol, m.p.* 212°.

The liquid (12 g.) was distilled from sodium and had then the following constants:— B.p., 55—56°/14 mm; d_{30}^{30} 0·8574; n_D^{30} 1·4660; $[\alpha]_D^{30}$ —71·9°. It was thought that this might be a mixture of camphene and tricyclene but the following experiments indicated that it was mainly camphene. Neither a nitrosochloride nor a bromide could be obtained; 4 c.c. in anhydrous ether (20 c.c.) when saturated with dry hydrogen chloride yielded camphene hydrochloride (3·9 g.; calculated, 4·3 g.) m.p. 145°. Oxidation with alkaline permanganate gave camphene-camphoric acid, m.p. 142°, while the portion unoxidised, when distilled over sodium and treated with hydrogen chloride, again gave camphene hydrochloride. It is thus probable that the liquid was an isomeric form of camphene which has been found in citronella oil (Gildemeister and Hoffmann, *loc. cit.*).

Alcohol fractions 4, 5, 6, 7 and 8 (Table I) were systematically refractionated under 12 mm. pressure as follows:—

TABLE III.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	$[R_L]_D$	Weight in grams	Yield per cent. on saponified oil
A	100—110°	..	1·4759	—30·0°	..	2·3	1·5
B	110—120	..	1·4789	—27·5	..	1·5	1·0
C	120—140	..	1·4819	—11·8	..	2·2	1·5
D	142—143	0·9128	1·4809	— 2·4	..	8·1	5·4
E	145—155	0·9448	1·4889	— 5·4	..	7·5	5·0
F	158—160	0·9601	1·4945	— 7·4	66·86	7·5	5·0
G	165	0·9674	1·4987	— 8·8	66·80	16·5	11·0
H	165—168	0·9690	1·4995	— 9·2	66·71	25·0	16·7
	Residue					15·0	10·0

Fractions A, B and C.—On cooling these fractions 0·3 g. of borneol was obtained melting at 202—204°. Tests for geraniol were negative.

Fraction D on treatment with sodium was almost completely decomposed and no distillate could be obtained. Sesquiterpenes are therefore not present, excepting possibly in very small quantity.

Fractions F, G and H.—The boiling points of these fractions indicate that they consist mainly of sesquiterpene alcohols, which thus comprise nearly 50 per cent. of the oil, and as they did not react with phthalic anhydride at 130-140° belong to the tertiary group. Attempts to prepare a hydrochloride or nitrosate were unsuccessful. Fraction H on being heated with selenium at 260° gave a hydrocarbon, b.p. 140—165°/12 mm. yielding cadalin picrate, m.p. 114-115° (mixed m.p. 114-115°), showing that the alcohols belong to the cadalin group of bicyclic sesquiterpene alcohols.

III.—ESSENTIAL OIL FROM LEAVES OF *THYMUS SERPYLLUM*, LINN.

By Jagjit Singh and B. Sanjiva Rao.

Many varieties of thyme are found along the Himalayán range. One growing in parts of the Punjab and Kashmir yields about 0·5 per cent. of an essential oil and was identified by C. E. Parkinson, Forest Botanist, to be *Thymus serpyllum*, Linn. It is a slender, much-branched shrub commonly known as the wild thyme and ranges from Europe through Northern Africa to Western and Central Asia. French oil of thyme is the most esteemed variety of thyme oil and is distilled from the herb *Thymus vulgaris*, Linn., its value being chiefly dependent on the 20–40 per cent. of thymol contained. In the south of France the oil not infrequently suffers admixture with the oil of *Thymus serpyllum*. As a result of previous examinations by Febve (*Compt. rend.*, 1881, **92**, 1290) and Jahns (*Arch. Pharm.*, 1880, **216**, 277) the presence of *p*-cymene, carvacrol and thymol in the latter oil has been definitely established, and it is known to contain only a very small amount of phenols; thus Jahns found only 1 per cent. and Buri (*Arch. Pharm.*, 1878, **212**, 485) 3 per cent. in the oils examined by them.

The Indian oil examined in these laboratories has constants somewhat different from the usually accepted values for *Thymus serpyllum* and approximating to those of *Thymus vulgaris* (Table I). The two samples examined contained 52·7 and 42 per cent. of phenols mainly carvacrol; tests for thymol gave negative results, the derivatives obtained being inseparable mixtures. Besides carvacrol, there is another phenol which has not been characterised, and in this connection it is of interest to note that when French thyme plants were grown in Germany they yielded oils containing carvacrol but no thymol. The neutral portion of the oil is rich in *p*-cymene (17 per cent.), and γ-terpinene also appears to be present. Phenylurethanes of the terpene alcohols were obtained melting at 134–135°, 127–129° and 108–115° which, although not in good agreement, indicate the probable presence of borneol and α-terpineol. In the higher boiling fractions of the oil, the sesquiterpene zingiberene has been definitely recognised, hitherto known to occur only in oils of ginger and *Curcuma zedoaria* (*this Journal*, 1928, **XIA**, 196).

The percentage composition of the oil is approximately as follows:—Phenols (mainly carvacrol), 53; *p*-cymene, 17; terpenes (γ-terpinene and others), 8; terpene alcohols, 5; sesquiterpenes (zingiberene and others), 4.

Experimental.

The oil was dark reddish brown, and on drying over anhydrous magnesium sulphate had the physical and chemical characters shown in Table I, which also gives the constants for the commercial French thyme oil and limits for *Thymus serpyllum* oil.

TABLE I.

	Thymus serpyllum (Indian)	*Thymus vulgaris* (French Thyme)	*Thymus serpyllum** (French)
d^{30}_{30}	0·9269	0·905—0·935	0·890—0·920
n^{30}_{D}	1·4968	1·4800—1·4950	..
$[\alpha]^{30}_{D}$	—4·8°	—0·5° to —4°	—10° to —20°
Phenols	52·7 per cent. (mainly carvacrol)	20 to 40 per cent. (mostly thymol)	Below 5 per cent.
Absorption with potassium metabisulphite	Nil	..	..

* E. J. Parry, *The Chemistry of Essential Oils and Artificial Perfumes*, 4th edition, 1921, 1, 246.

The oil is thus richer in phenols than that obtained from *Thymus serpyllum* grown in other parts of the world and the constants are comparable with those for French thyme oil.

Phenols.—The oil (500 g.) was washed with aqueous sodium carbonate and the phenols isolated by shaking with 4 per cent. sodium hydroxide on a water bath, acidifying the alkaline extract with dilute sulphuric acid and extracting the liberated phenols (52·7 per cent.) with ether. The ethereal solution was washed with water, dried and the ether distilled; the product (240 g.) on repeated fractionation with a 4–pear Young's column under 32 mm. pressure gave the following fractions:—

TABLE II.

Fractionation of Phenols.

Fraction	B.P.	d^{30}_{30}	n^{30}_{D}	Weight in gms.	Yield per cent. on total phenols	Yield per cent. on original oil
1	127—130°	0·9613	1·5118	5·0	2·1	1·1
2	130—132	0·9701	1·5158	5·0	2·1	1·1
3	132—134	0·9710	1·5162	6·5	2·7	1·4
4	134	0·9719	1·5171	6·5	2·7	1·4
5	134	0·9719	1·5172	7·0	3·1	1·5
6	134—136	0·9719	1·5172	10·0	4·1	2·2
7	135—136	0·9719	1·5173	10·0	4·1	2·2
8	,,	0·9727	1·5175	10·8	4·4	2·4
9	,,	0·9727	1·5178	9·0	3·7	2·0
10	,,	0·9727	1·5178	77·0	33·3	16·9
11	,,	0·9728	1·5178	7·0	3·1	1·5
12	,,	0·9732	1·5178	54·0	22·4	11·9

The constants for pure thymol and carvacrol being as follows:—

	B.P.	d^{30}_{30}	n^{30}_{D}
Thymol	132°/32 mm.	0·965	1·5186 (superfused)
Carvacrol	136°/32 mm.	0·972	1·5200

It is evident that not more than a small proportion of thymol could be present; confirming this the fractions were cooled for two days at –5° without solidification. According to Sage and Dalton (*Perf. and Essent. Oil Rec.*, 1924, **15**, 315) mixtures of pure thymol and carvacrol containing 5, 10 and 20 per cent. thymol melt at about 1°, 3° and 9°, respectively, so that thymol, if present, did not exceed 5 per cent. in any fraction. The first two fractions alone were likely to contain this phenol and as they formed only 2·2 per cent. of the whole oil it follows that the amount of thymol was less than 0·1 per cent. The physical characters of the fractions showed that these were not pure carvacrol, but it was not found possible to identify other phenols. Attempts to detect thymol in the fractions by means of colour reactions applicable to thymol, carvacrol and their mixtures were unsuccessful due perhaps to the presence of a new phenol.

Phenylurethanes.—Fractions 1, 6 and 12 (5 g. each), phenyl *iso*cyanate (4·6 g.) and petroleum (b.p. 120–150°, 30 c.c.) were boiled in an acetylation flask during two hours. The cooled mixture deposited crystals which were recrystallised from petrol and ethyl alcohol in succession until the melting point was constant. The phenylurethane from fraction 12 showed no depression with carvacrol phenylurethane (m.p. 138°).

Nitrosophenols.—To 5 c.c. each of fractions 2, 6 and 12 dissolved in ethyl alcohol (25 c.c.) was added concentrated hydrochloric acid (25 c.c.), cooled to zero and gradually treated with a saturated solution of sodium nitrite (3·6 g.). On pouring into water a light yellow bulky solid was obtained which was recrystallised from benzene.

Benzoyl derivatives of nitrosophenols.—The nitrosophenol (3 g.) obtained from fractions 2, 6 and 12, was dissolved in 10 per cent. sodium hydroxide solution (10 c.c.) and benzoyl chloride (3·5 g.) added with shaking; the product separated as orange plates, and was recrystallised from alcohol. Table III shows the melting points of the derivatives obtained.

TABLE III.

Melting Points of Phenol Derivatives.

	Phenylurethanes	Nitroso derivative	Benzoyl derivatives of nitrosophenols
Fraction 1, 2	(1) 132—136°	129°	(1) 93—98°
	(2) 125—134		(2) 77—85
	(3) 105—115		
Fraction 6	137	131	(1) 74—80
			(2) 78—85
Fraction 12	138	152	(1) 73—76
	..	..	(2) 85
Thymol	106—107	160—164	109·5
Carvacrol	138	153	87, 85

Fraction 1 appears to be a mixture of carvacrol and thymol, although the latter could not be detected, and subsequent fractions become richer in carvacrol. Fraction 6 gives a pure phenylurethane of carvacrol but the nitroso- and benzoyl derivatives appear to be mixtures. Fraction 12 is pure carvacrol, all its derivatives having correct melting points.

To effect a separation of the phenols (1) ether extraction of alkaline solutions of varying strength, and (2) steam distillation were tried, but no separation could be effected. Shaking with a solution of normal potassium phosphate and examining the dissolved and undissolved phenols, gave a phenylurethane m.p. 103° from the dissolved portion, and this may be crude thymol phenylurethane.

Neutral oil.— After removing phenols by sodium hydroxide the residual oil was washed with water and dried over anhydrous magnesium sulphate when it had the following constants:—d^{30}_{30} 0·9017, n^{30}_{D} 1·4781, $[\alpha]^{30}_{D}$—7·65°, ester value 34·0, acetyl value 47·5. The oil (150 g.) was saponified with *10 per cent. alcoholic potash, diluted with water and extracted with ether.* The ethereal solution was dried, the ether distilled and the residual oil fractionated under 50 mm. pressure with a 3-bulb Le-Bel column giving the results shown in Table IV.

TABLE IV.

Fraction	B.P.	d^{30}_{30}	n^{30}_{D}	$[\alpha]^{30}_{D}$	Weight in grams	Yield per cent. on original oil
1	below 80°	..	1·4708	..	..	..
2	80— 90	0·8449	1·4713	— 3·8°	19·0	6·0
3	90— 93	0·8473	1·4749	— 2·0	51·0	16·0
4	95—100	0·8540	1·4765	— 1·4	15·5	4·8
5	100—105	0·8684	1·4770	— 2·6	6·5	2·0
6	105—110	0·8744	1·4770	— 3·4	3·8	1·2
7	110—120	0·9019	1·4792	— 7·3	2·2	0·7
8	120—130	0·9217	1·4821	—10·5	5·5	1·7
9	130—140	0·9319	1·4888	—14·6	13·5	4·2
10	140—150	0·9227	1·4951	—22.8	9·5	3·0
11	150—160	0·8973	1·4925	—41·5	8·5	2·6
12	160—175	0·9325	1·5002	—23·1	3·5	1·1
	Residue and loss				11·5	3·6

Fractions 2, 3, 4 and 5 which consist of the greater part of the terpenes present in the oil were repeatedly fractionated with a Young's column under 50 mm. pressure, the results being shown in Table V.

TABLE V.

Fractionation of Terpenes.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in gms.	Yield per cent. on original oil.
A	62—76°	..	1·4606	..	0·6	0·2
B	76—78	0·8436	1·4663	—7·3°	3·8	1·2
C	78—83	0·8436	1·4691	—5·5	7·0	2·2
D	84—88	0·8436	1·4720	—2·6	2·5	0·8
E	88—90	0·8444	1·4742	—2·4	19·8	6·2
F	90—91	0·8461	1·4751	—0·9	8·5	2·7
G	91—92	0·8496	1·4765	—0·7	30·5	9·6
H	92—93	0·8540	1·4770	—0·7	6·0	1·8

No ketones or aldehydes were detected in any of the fractions. A low boiling alcohol appeared to be present in A and B but no phenylurethane could be prepared. Attempts to prepare a solid hydrochloride from B, D and H, and a nitrosochloride, nitrosite or nitrosate from C, E and H were unsuccessful.

Fraction E (*γ-terpinene.*).— Fraction E (10 c.c.), potassium permanganate (60 g.), potassium hydroxide (16 g.), ice (500 g.) and water (500 g.) were mixed in a bottle and shaken mechanically. The unattacked terpene (3·5 c.c.) was distilled in steam. The filtrate after removal of manganese dioxide was evaporated to dryness and the residue extracted with alcohol. A small amount of a solid was obtained which melted at 231–234°, probably the erythritol $C_{10}H_{16}(OH)_4$ of γ-terpinene, which melts at 237–238°. The mother liquor from the erythritol on acidifying with dilute sulphuric acid yielded *p*-hydroxy*iso*propylbenzoic acid, m.p. 156–157° derived from *p*-cymene (see fraction G).

Fraction G (p-*cymene*).—Fraction G (5 c.c.) with potassium permanganate (20 g.) and water (400 c.c.) were boiled under reflux for two days and shaken occasionally. The potassium salts obtained by evaporation were boiled with alcohol and filtered. The soluble potassium salt on acidifying gave an acid m.p. 156–157° (equivalent 179·2) on recrystallisation from alcohol. A specimen of *p*-hydroxy*iso*propylbenzoic acid prepared from a sample of pure *p*-cymene had m.p. 156–157° and gave no depression when mixed with the acid obtained from fractions F, G and H confirming the presence of *p*-cymene.

Fractions 6 and 7 did not yield a phenylurethane or nitrosochloride.

Fractions 8, 9 *and* 10 yielded solid phenylurethanes on boiling with phenyl*iso*cyanate in petroleum solution, but all the derivatives were found to be complex mixtures melting over a wide range. On fractional crystallisation from petrol three crops were obtained with melting points 134–135°, 127–129° and 108–115°. An attempt to confirm the presence of α-terpineol by

preparing the nitrosite was not successful. It is probable that borneol (phenylurethane, m.p. 138°) and α-terpineol (phenylurethane, m.p. 112–113°) are present in the fractions.

Fraction 11 (*zingiberene*).—On repeated distillation over sodium which reacted with a considerable proportion of the oil, 6 g. were obtained having the following constants:—B.p. 157–162°/45 mm., d_{30}^{30} 0.8886, n_D^{30} 1·4945, $[\alpha]_D^{30}$ —52°, $[R_L]_D$ 66.9. Zingiberene has b.p. 164–165°/45 mm., d_{30}^{30} 0·8639, n_D^{30} 1·4916, $[R_L]_D$ 68·4. The constants indicate the presence of a bicyclic sesquiterpene besides zingiberene. The fraction gave a nitrosochloride with amyl nitrite and hydrochloric acid melting at 95–96° after crystallisation from methyl alcohol and unchanged on mixing with zingiberene nitrosochloride. A nitrosate (m.p. 86–87°) was also obtained confirming the presence of zingiberene. In fraction 10, the fall in density and rise in refractive index and rotation, is due to admixture with zingiberene whose presence has been shown in fractions 11 and 12. The content of zingiberene in the oil is approximately 0·9, 2·0, 0·5 per cent. respectively in fractions 10, 11 and 12, or 3·4 per cent. in the whole oil.

Fraction 12 was apparently a mixture of sesquiterpenes and a small quantity of sesquiterpene alcohols. Zingiberene nitrosochloride was also obtained from this fraction. There was no reaction with phthalic anhydride at 130–140°, showing the absence of primary and secondary alcohols.

IV.—ESSENTIAL OIL FROM LEAVES OF *CINNAMOMUM ZEYLANICUM*, BREYN.

By (the late) V. P. Shintre and B. Sanjiva Rao.

Cinnamomum Zeylanicum, Breyn, is an interesting example of a plant in which the leaves, bark and root yield oils differing markedly in composition. The bark is among the longest known and used spices, and the leaf oil has been a valuable commercial product on account of its eugenol content. The tree is not cultivated on any appreciable scale in India, but is found wild in the Western Ghats. *Cinnamomum Zeylanicum* exists in a number of varieties and according to E. M. Holmes (*Perf. and Essent. Oil Rec.*, 1916, **7**, 41), these are difficult to classify, some botanists regarding them as distinct species. As the tree can grow from sea-level up to the highest elevation it is possible the varieties are due to local environment and soil. In view of the fact that seven or eight well-defined varieties have been observed in the moist forests of the Western Ghats (Beddome, *Flora Sylvatica for Southern India*, 1872, 262) it seemed of interest to make a chemical examination of the volatile oils obtained from them. The present paper deals with an oil distilled from a variety growing in the neighbourhood of Chamarajnagar in Mysore, which appears to be fairly common in the hilly parts of Mysore and was identified as *Cinnamomum Zeylanicum*, Breyn, by the botanist at Coimbatore. The leaves on steam distillation yielded a yellow oil which differed appreciably from the cinnamon leaf oils of commerce, the eugenol content being only 15 per cent. After fractional distillation *l*-α-pinene and *l*-β-phellandrene were identified in the terpene fraction. A tertiary alcohol was found in the fraction boiling at 140–160°/100 mm. which yielded *trans*terpin on treatment with sulphuric acid. This indicates the presence of γ-terpineol, a substance only once previously recorded as existing in a natural essential oil, that of *Cupressus torulosa* (Simonsen, *Indian Forest Records*, 1923, **10**, 1). The sesquiterpene fractions gave several derivatives of α-caryophyllene, the alcohol (m.p. 95–96°), nitrosochloride (171°) and nitrosate (163°) being isolated. The rotation of these fractions was low and it is probable that a considerable proportion of the inactive form of α-caryophyllene was present. Additional evidence for this was found in the fact that no solid dihydrochloride could be obtained, this derivative being a liquid in the case of inactive α-caryophyllene (Deussen, *J. pr. Chem.*, 1929, **120**, 141; Chapman, *J.C.S.*, 1928, **127**, 785).

The approximate percentage composition of the oil is as follows:—

Terpenes (*l*-α-pinene and *l*-β-phellandrene), 4; terpene alcohols (γ-terpineol and others), 3; eugenol, 15; sesquiterpenes (mainly α-caryophyllene), 62; sesquiterpene alcohols, 7.

Experimental.

On steam distilling 639 lbs. of the fresh leaves (moisture 46·8 per cent.) 493 g. of lemon-yellow oil was obtained, or 0·25 per cent. calculated on the

dry leaves. The oil was dried over anhydrous magnesium sulphate and was found to have the constants given in Table I, which also shows some values recorded by other observers.

TABLE I.

	Bangalore	Normal oils*	Glichitch†	Schimmel‡
d_{15}^{15}	0·9223	1·043–1·066	1·0650	1·0183
n_D^{20}	1·5070	1·530–1·540	1·5438	1·5220
α_D^{20}	-2·9°	-0°10′ to +2°35′	-1·6°	+16·4°
Phenols per cent.	15	70–90	70	78

* *Bull. Imp. Inst.*, 1921, **19**, 324.
† *Les Parfums de France*, 1924, 66.
‡ *Schimmel's Report*, 1892, 58.

The low density, refractive index and phenol-content indicate that the oil is not of the normal quality.

The oil (423 g.) was washed with dilute aqueous sodium carbonate to remove free acids and then with 5 per cent. potassium hydroxide to remove the phenols. The non-phenolic portion was extracted with ether, washed free from alkali and dried with anhydrous magnesium sulphate. The oil (340 g.) freed from ether was distilled at 100 mm. pressure, using a 4–pear Young still-head, the results being recorded in Table II.

TABLE II.

Fractionation of the Oil.

Fraction	B.P.	d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$	Weight in grams	Yield per cent. on original oil
1	100—120°	0·8420	1·4702	—30·8°	5·0	1·3
2	120—135	0·8437	1·4706	—30·5	7·0	1·8
3	135—140	0·8457	1·4718	—25·9	1·5	0·4
4	140—160	0·8570	1·4776	—19·0	7·0	1·8
5	160—170	0·8729	1·4846	—10·1	8·0	2·0
6	170—180	0·8998	1·4920	— 4·9	24·0	6·0
7	180—182	0·9064	1·4982	— 1·7	143·0	35·9
8	182—186	0·9117	1·4988	— 1·5	24·0	6·0
9	186—200	0·9250	1·5004	— 1·3	31·0	7·8
10	200—210	0·9518	1·5048	..	9·0	2·3

The first three fractions were redistilled over sodium at 100 mm. and the following fractions were obtained :—

		d_{30}^{30}	n_D^{30}	$[\alpha]_D^{30}$
A	95—100°	0·8474	1·4660	—40.6°
B	100—105	0·8509	1·4698	—32.5

l-*α-Pinene and* l-*β-phellandrene*:—Both fractions yielded a nitrosochloride, m.p. 103—104° which on treatment with piperidine gave a nitrolpiperidide, m.p. 118—119° showing the presence of *l*-α-pinene.

When treated with sodium nitrite and acetic acid in petrol ether solution a fair yield of a nitrite was obtained from fraction B. It was recrystallised from acetone and melted at 101-102°; 0.1199 g. dissolved in 10 c.c. chloroform had α_D= -1.7, $[\alpha]_D$ = -142°. The more soluble nitrite was obtained as an oil and could not be crystallised; *l*-β-phellandrene nitrite, according to Wallach (*Annalen*, 1904, **336**, 43), melts at 102° and has $[\alpha]_D$ = -159.3°.

Fraction 4 (*γ-terpineol*).—No semicarbazone or urethane could be obtained from this fraction, nor did it react with phthalic anhydride showing that it is a tertiary alcohol. On shaking (5 c.c.) at room temperature with 1 per cent. dilute sulphuric acid (200 c.c.) for 20 hours and extracting with ether, an oil was obtained which solidified on standing. Recrystallised from ethyl acetate it melted at 156°, undepressed by admixture with authentic *trans*terpin. The formation of *trans*terpin indicates the presence of γ-terpineol in the original oil.

Fraction 5 was probably a mixture of terpene alcohols and sesquiterpenes.

Fractions 6, 7, and 8.—These were thrice refractionated, the final distillation under 6 mm. pressure over sodium giving the following fractions:

TABLE III.

Fraction	B.P.	d^{30}_{30}	n^{30}_D	$[\alpha]^{30}_D$	Weight in grams	Yield per cent. on original oil	C.	H.
a	105—108°	0·8794	1·4908	..	3·5	0·9	..	..
b	108—110	0·8835	1·4955	—7·3°	82·0	20·6	88·1	11·9
c	113—115	0·8882	1·4978	—1·2	77·0	19·3	88·2	11·9
d	115—120	0·8968	1·5018	+6·1	17·5	4·4	..	..

Fractions a, b and *c* (*α-caryophyllene*).—On treatment with dry hydrogen chloride, neither fraction gave a solid dihydrochloride, indicating the absence of any large proportion of active α-caryophyllene.

By Bertram and Walbaum's method no solid alcohol could be obtained, but treatment with a mixture of absolute ether and sulphuric acid monohydrate (Asahina and Tsukomoto, *J. Pharm. Soc. Japan*, 1922, June) resulted in a good yield. The product crystallised from alcohol in silky needles which melted at 95-96° remaining unchanged when mixed with an authentic specimen of caryophyllene alcohol. The phenylurethane prepared in the usual manner melted at 136-137° (Found: N, 4·0: $C_{22}H_{29}O_2N$ requires N, 4·1 per cent.).

Both *b* and *c* gave good yields of a crystalline nitrosochloride, m.p. 171°, and a crystalline nitrosate m.p. 162-163°. Both substances yielded when treated with benzylamine a derivative melting at 128°, confirming the presence of α-caryophyllene.

Fraction 10 (*sesquiterpene alcohols*).—Analysis (Found: C, 82·0; H, 11·2; $C_{15}H_{24}O$ requires C, 81·8; H, 10·9 per cent.) showed the fraction to be mainly sesquiterpene alcohol. It did not react with phthalic anhydride and therefore did not contain primary or secondary alcohols. No derivatives were obtained.

Phenols were liberated by bubbling carbon dioxide into the potassium hydroxide extract of the oil. They were extracted with ether and after removing ether, distilled under diminished pressure, the yield being 14·7 per cent. (b.p. 115–118°/8 mm. d_{30}^{30} 1·0604, d_{15}^{15} 1·0703, n_D^{15} 1·5432). Eugenol has b.p. 123°/12 mm, 118–119°/8 mm, d_{15}^{15} 1·072, n_D^{15} 1·5439 (*Ber.*, 1890, **23**, 262), so the constants are in good agreement. A benzoyl derivative melting at 69–70° was obtained when the phenol was treated with benzoyl chloride in presence of potassium hydroxide. The phenylurethane prepared in the usual manner melted at 94–95°, unaltered on admixture with a pure specimen of eugenol phenylurethane.

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ORGANIC MANURE FROM SEWAGE, TOWN REFUSE AND WASTE VEGETATION.

By J. Jagannatha Rao and V. Subrahmanyan.

The problem of utilising waste has received attention from the earliest times. In ancient China and Japan, conversion of different forms of farm and domestic waste into manures was a recognised item of agricultural practice (King, Farmers of Forty Centuries, 1911). In more recent times, various other methods such as feeding to hogs, extraction for fat (*Amer. J. Pub. Health*, 1912, **2**, 937; *J. West. Soc. Eng.*, 1929, **34**, 102), incineration for development of energy (*Eng. News Rec.*, 1923, **91**, 844; *J.S.C.I., Rev.*, 1927, **5**, 119), and the manufacture of cement, building stones (Kershaw, B. P., 149,033 of 1919) and various types of glasses (*Compt. rend.*, 1924, **178**, 1161) have been developed, but most of them are inapplicable to the commoner types of refuse which contain very little fat or other extractable matter and are not readily combustible (Clemesha, Sewage Disposal in the Tropics, 1910, 230). In view of the above and the readiness with which they undergo decomposition in presence of suitable chemical and biological starters, composting has been recognised to be the most suitable method of utilising waste materials particularly in the tropics where the soils are naturally deficient in carbonaceous matter and respond readily to treatment with organic manures.

Scientific study of the subject did not, however, receive much attention until Russell and Richards (*J. Agric. Sci.*, 1917, **8**, 495) and later, Richards and Hutchinson (*J. Min. Agric.*, 1921, **28**, 398) investigated the nature of the transformations attending the decomposition of straw in presence of different forms of nitrogen. Subsequent contributions by Rege (*Ann. Appl. Biol.*, 1927, **14**, 1), Norman (*Biochem. J.*, 1929, **23**, 1353, 1367; *Ann. App. Biol.*, 1930, **17**, 575) and Jensen (*J. Agric. Sci.*, 1928, **19**, 71) in England, Waksman, Starkey and their co-workers in America (*Soil Sci.*, 1927, **24**, 275, 317; *Ibid.*, 1928, **26**, 113, 155, 239; *Ibid.*, 1929, **27**, 271, 319, 355, 389, 433; **28**, 55; *J. Amer. Sci. Agron.*, 1929, **21**, 795) and Krantz, Gerlach, Ruschman, Löhnis and others in Germany (*Nature*, 1927, **122**, 339; *Z. pflanz. Dung.*, 1928, **B8**, 303, *Ibid.*, 1929, **A13**, 208; *Ibid.*, **B8**, 529; *Ibid.*, **B9**, 268; *Zentralbl. Bakt.*, 1928, **II 75**, 405; *Ibid.*, 1929, **II 77**, 216) have helped to throw further light on the decomposition of cellulosic materials in the manure heap.

In India, a considerable amount of work on the preparation of organic manures from waste materials was carried out by Hutchinson and his assistants at Pusa (*Agric. Res. Inst., Pusa*, Bull., 1914, **46**, *et. seq.*), Howard and Wad at Indore (The Waste Products of Agriculture, 1931), Carberry and Finlow in

Bengal (*Agric. J. India*, 1928, **23**, 80), Clarke and his associates at Shajahanpur (*Proc. Indian Sci. Cong.*, 1930, **17**, 23), Viswanath and his co-workers at Coimbatore, Coleman and his staff in Mysore and Fowler and his students at Bangalore, Nasik and Cawnpore (*Agric. J. India*, 1930, **25**, 369).

Although a considerable measure of success has already been achieved, yet the precise chemical and biological mechanism of the decomposition changes, particularly in heterogeneous masses like town refuse, has not yet been fully investigated. Moreover, further work has to be carried out on the standardisation of conditions for the (*a*) most efficient conversion of the different organic components into plant nutrients, (*b*) suppression of foul odours and destruction of putrefactive and pathogenic organisms and harmful insects, (*c*) fixation of atmospheric nitrogen, evidence for which has been adduced by some of the previous workers (*e.g.*, Howard and Wad, *loc. cit.*; Fowler, *loc. cit.*), (*d*) minimisation of loss of organic matter and preservation of nitrogen both during and after completion of the decomposition, and (*e*) application of the manure to land with and without addition of mineral fertilisers. The present investigation was, therefore, undertaken to elucidate the above and related problems.

Experimental.

Preliminary trials in pots.—To standardise conditions for the experimental heaps, trials were made with powdered town refuse in glazed, non-draining, earthenware pots. The refuse (½ lb.) was loosely packed and the pots divided into 3 sets which were treated as follows:—(*a*) Control (untreated), (*b*) mixed uniformly with chemicals (ammonium sulphate, 7 g.; rock phosphate containing 26 per cent. P_2O_5, 2 g.; and burnt lime 8 g.), (*c*) treated with chemicals as in *b plus* 50 c.c. of activated sludge (15 per cent.) as biological starter. The contents of the different sets were moistened with varying proportions of raw sewage or effluent from the activated sludge tank and the decomposition changes followed from day to day. It was soon observed that the finely divided refuse was exceedingly difficult to work, owing to its tendency to cake. Aeration was seriously hampered and if the material was not stirred frequently decomposition slackened with attendant bad smell, flies and mosquitoes.

Putrefactive organisms and harmful insects were suppressed by spraying the contents of the pots with small amounts (50 c.c.) of solutions containing (*a*) bleaching powder (aqueous suspension containing 0·3 per cent. chlorine), or (*b*) dilute Bordeaux mixture (containing $CuSO_4$ $5H_2O$, 0·06 per cent. and lime 6·0 per cent.). It was also noted that the decomposition was most rapid in pots treated with activated sludge and subsequently sprayed with copper-lime mixture, the decomposing materials developing a characteristic dark colour; while a microscopic examination of the cellulosic fibres showed that they were undergoing more rapid physical disintegration.

In view of the foregoing observations, further trials were made with specimens of whole refuse after treating it with chemicals and activated sludge

as in experiment *c*; the decomposition, as indicated by loss in dry matter, proceeded more rapidly under such conditions than when the refuse was finely divided. No foul odours were evolved and there was less need for either stirring or spraying with insecticides and fungicides. The disintegration of fibrous materials, however, appeared to proceed faster in presence of copper-lime mixture than otherwise.

In order to determine whether the increased darkening observed in the case of specimens sprayed with the copper mixture was due to more intensive humification, or to some product of reaction of either copper or lime, or of both combined with some of the decomposing materials, specimens were treated as follows:—(*a*) Control (untreated), (*b*) sterilised by autoclaving and sprayed with (1) suspension of lime, (2) solution of copper sulphate, and (3) copper-lime mixture. It was observed that specimens *b* (2) and (3) were distinctly darker than the others and that the colour tended to deepen on standing, showing that the colour change was due to a chemical reaction of copper and not to increased humification.

Trials in heaps.—Refuse was collected from busy streets in Bangalore City and was heterogeneous, containing non-decomposable or resistant glass, tins, bones, feathers, etc., with readily putrefactive kitchen waste and night-soil.

Sampling for analysis was done by collecting representative specimens from different parts of the mixed heaps, combining the small lots thus obtained, then crushing them to fine division, and finally collecting specimens as for soil examination. The average percentage decomposition of the oven-dried material as determined by the A. O. A. C. (1925) methods was as follows:—Loss on ignition, 64·7; total nitrogen, 0·69; total phosphorus (as P_2O_5), 0·40; total potash (as K_2O), 0·19. The daily collections spread over a number of days were first mixed together, then divided into 16 small heaps each weighing about half a ton. The heaps were piled on plots of land 12 ft. square separated from each other by a drain 1 ft. wide and 1 ft. deep. They were then treated with different chemical and biological starters (Table I), the actual quantities of the different ingredients being so adjusted as to correspond to 0·1 per cent. of nitrogen, and 0·03 per cent. each of K_2O and P_2O_5 on the original dry weight of the material. The heaps were moistened from day to day with either raw sewage or effluent from the activated sludge tank, 60 gallons of each fluid being sprayed in the morning and evening of each day. The heaps were thoroughly stirred three times a day to ensure complete aeration.

After the third day, the heaps emitted unpleasant odours and harboured numerous flies, mosquitoes and other insects which were effectively suppressed by spraying with thin suspensions of either copper-lime mixture (*loc. cit.*) or bleaching powder suspension (*loc. cit.*), no more than 4 litres of each being required per heap: dusting with cyanamide was not so satisfactory, and the foul odour persisted although the treatment was repeated at frequent intervals. The scheme of treatments may be stated briefly as follows;—

TABLE I.

Heap No.	Nitrogen as	Phosphoric acid as	Insecticide
1	Act. sludge + R. S.	Rock phosphate	Bleaching powder
2	CaNCN + do.	do.	Bordeaux mixture
3	R. S. only	do.	do.
4	R. S. + CaNCN	do.	CaNCN
5	$(NH_4)_2SO_4$ + R. S.	Superphosphate	Bordeaux mixture
6	$NaNO_3$ + R. S.	do.	do.
7	Effl. only	Nil	Nil
8	$NaNO_3$ + Effl.	Rock phosphate	Bleaching powder
9	$(NH_4)_2SO_4$ + do.	do.	Bordeaux mixture
10	do. + do.	do.	do.
11	CaNCN + do.	do.	Bleaching powder
12	$NaNO_3$ + do.	Superphosphate	do.
13	$NaNO_3$ + CaNCN + Effl.	do.	CaNCN
14	$(NH_4)_2SO_4$ + Effl.	do.	Bleaching powder
15	Act. sludge + do.	do.	do.
16	CaNCN + do.	do.	CaNCN

R.S.=Raw sewage; Effl.=Effluent from the activated sludge tank. All the heaps were treated with potassium sulphate corresponding to a potash (K_2O) content of 0·03 per cent.

The commercial specimens used for the above treatments were analysed for the percentages of their active constituents:—K_2O in potassium sulphate, 38·3; P_2O_5 in superphosphate, 29·3; P_2O_5 in rock phosphate, 26·0; N in ammonium sulphate, 20·89; N in sodium nitrate, 16·2; N in cyanamide, 18·1; CaO in burnt lime 63·5. The raw sewage used for spraying had an average nitrogen content of 4 parts per 100,000 and the effluent, 2 parts per 100,000.

Loss of organic matter during decomposition.—This was determined indirectly as follows. Representative specimens of the decomposing material were collected at stated intervals and the ash-contents determined on the oven-dry basis. Hence the corresponding changes in organic matter were calculated, assuming that during decomposition the total ash constituents remain unaltered and that the increase in ash percentage noticed at the time of observation is really due to a corresponding loss in organic matter. Thus if X represents the initial ash percentage, then the corresponding amount of organic matter (as represented by loss on ignition) would be 100—X: if at the time of observation the ash percentage is represented by Y, and if Z be the unknown loss in organic matter, then,

$$\frac{100\,[\,(100-X)-Z\,]}{100-Z}=100-Y$$

which, on solving, gives

$$Z=\frac{100\,(Y-X)}{Y}$$

in which both X and Y are known. Table II represents the figures thus determined for the different heaps.

TABLE II.

Heap No.	Percentage loss in organic matter in		
	20 days	35 days	70 days
1	11·3	13·4	20·1
2	13·0	15·5	19·5
3	12·4	14·7	21·2
4	10·4	13·6	20·8
5	11·7	14·8	20·3
6	13·0	15·9	21·2
7	9·7	11·3	14·9
8	10·6	13·9	21·2
9	12·6	15·3	20·1
10	11·9	14·8	21·0
11	11·3	14·1	19·7
12	11·0	13·0	19·7
13	10·8	13·2	21·5
14	10·3	13·9	20·1
15	11·0	13·4	21·2
16	10·6	13·0	20·6

Analyses (unrecorded) of representative specimens at the end of 60 days had shown that the loss in organic matter was about 20 per cent. from every one of the heaps with the exception of No. 7. Since 10 days later, the organic matter was found to have suffered no further loss, it was concluded that further decomposition, if any, would be very slow and the corresponding loss of organic matter almost negligible. Further spraying with sewage was therefore stopped, the contents of the heaps air-dried and the material thus obtained used for further chemical analyses and vegetation experiments.

It was noted in the case of the heap (No. 7) that had been treated with effluent alone that most of the cellulosic materials remained intact, rigidity of the fibrous substances suggesting very poor decomposition. In the case of the others the physical breakdown had proceeded very much farther; even those substances which retained their shape crumbled readily on crushing. The effect was most marked in heaps 1 and 15 which had previously been treated with activated sludge. Those specimens were darker than the others and were readily friable on gentle pressure; although their organic contents were the same their physical condition suggested that these would be more readily available for plant nutrition than the others.

Chemical composition of the preparations.

Nitrogen distribution:—This was determined on the oven-dry basis according to the A.O.A.C. (1925) methods, and the results shown in Table III.

TABLE III.

Heap No.	Nitrogen per cent.			
	Total	Albuminoid	Ammonia	Nitrate
1	1·70	0·57	0·17	0·24
2	1·17	0·55	0·15	0·20
3	1·21	0·35	0·04	0·14
4	0·96	0·35	0·06	0·11
5	0·96	0·43	0·14	0·15
6	1·08	0·39	0·04	0·15
7	0·81	0·44	0·03	0·05
8	1·20	0·36	0·06	0·10
9	1·12	0·38	0·09	0·23
10	0·92	0·29	0·03	0·07
11	0·90	0·31	0·05	0·07
12	1·02	0·48	0·04	0·09
13	0·96	0·41	0·08	0·09
14	0·87	0·30	0·05	0·06
15	1·17	0·47	0·05	0·23
16	0·90	0·38	0·05	0·07

Considering the heterogeneity of the materials and the inevitable error in sampling, the differences between the nitrogen contents of most of the heaps are not significant. The two heaps originally treated with activated sludge (1 and 15) were exceptions and contained larger quantities than the others. The heap that had received its nitrogen from the effluent alone was apparently the poorest, but the percentage was not very much lower than those of some others. The heaps sprayed with raw sewage are on the average slightly richer in nitrogen than those receiving effluent, but the difference is not very considerable.

Distribution of nitrogen into the different forms shows no striking relation traceable to the previous treatment except in the case of No. 1 (activated sludge) which is richer in all the constituents than the others: nor could a special advantage be claimed for any of the nitrogenous starters excepting activated sludge.

Nitrogen balance.—Previous workers have reported losses of nitrogen ranging from 10–40 per cent. of the total either during the preparation or storage of organic manures. The extent to which such losses occur during either fully ærobic conditions or strictly anærobic ones is still obscure (Barrit, *Biochem. J.*, 1931, **25**, 1965). In composting experiments it is very difficult to maintain exclusively ærobic or anærobic conditions, and there is generally a combination of both; material either exposed to air or near the surface is under partly ærobic conditions while the interior, particularly

when the heaps are not turned frequently, would tend to be anærobic. In the present investigation the conditions were maintained as nearly ærobic as possible by turning the heaps at least three times a day.

To determine the effect of different treatments, as also the related air conditions on the nitrogen balance of the decomposing heaps, the total quantities of nitrogen originally present in, subsequently added to and finally present in the various heaps were calculated, and the ultimate gain or loss estimated (Table IV).

TABLE IV.

TOTAL NITROGEN.

Heap No.	Weight of heap in lbs.		Total Nitrogen											
	Initial	Final	Originally in the refuse		Added as chemicals and/or sprayed as sewage		Expected in the final product		Found in the final product		Gained (+) Lost (—)			percentage
			lb.	oz.	lb.	oz.	lb.	oz.	lb.	oz.		lb.	oz.	
1	973	777	7	1	4	5	11	6	13	4	(+)	1	14	+16.5
2	744	599	5	7	4	3	9	10	7	0	(—)	2	10	—27.3
3	1,086	856	6	11	4	7	11	2	10	6	(—)	0	12	— 7.0
4	1,534	1,215	9	6	4	13	14	3	11	11	(—)	2	8	—17.6
5	1,005	801	7	5	4	6	11	11	7	11	(—)	4	0	—34.2
6	1,013	798	7	6	4	6	11	12	8	10	(—)	3	2	—26.6
7	1,370	1,166	8	5	3	0	11	5	9	7	(—)	1	14	—16.6
8	700	552	5	1	2	5	7	6	6	10	(—)	0	12	—10.2
9	708	566	5	2	2	6	7	8	6	5	(—)	1	3	—15.8
10	1,205	952	8	12	2	12	11	8	8	12	(—)	2	12	— 23.9
11	990	795	7	2	2	9	9	11	7	2	(—)	2	9	—26.5
12	753	605	5	7	2	6	7	13	6	3	(—)	1	10	—20.7
13	863	675	6	6	2	7	8	13	6	8	(—)	2	5	—26.2
14	1,502	1,200	10	14	3	0	13	14	10	7	(—)	3	7	—24.8
15	690	544	5	1	2	5	7	6	8	0	(+)	0	10	+ 8.5
16	984	781	7	1	2	9	9	10	7	0	(—)	2	10	—27.3

It will be seen that there was distinct loss (7·0—34·2 per cent.) of nitrogen from the majority of heaps, and this could not be related to the treatment; but there appears to be a marked loss from all heaps receiving mineral starters irrespective of their chemical nature.

The significance of the foregoing observations will be appreciated on realising that the organic manures under investigation are worth at least Rs. 5 (*7s. 6d.*) per ton, and since they contain (approximately) 1 per cent. nitrogen, the latter represents about As. 4 (4½*d.*) per pound. The losses range from 12 oz. to 4 lbs. of nitrogen and correspond to As. 3. to Re. 1 per heap. As the average weight per heap is about 1000 lbs. and the corresponding loss of nitrogen, 2 lbs., it may be reckoned that the equivalent monetary loss will be Rs. 1-4-0 (about 2*s.*) on the preparation of each ton of manure. The extent to which town refuse becomes available depends on the occupation and habits of the citizens and the efficiency of collection. It may, however, be assumed that the daily collections will correspond to about 1 ton per 1000 of population. About one-sixth of India's population live in towns or villages with

organisation for the collection of refuse, so that the annual collections, and the manure that could be prepared from them may be rated at 20 and 15 million tons, respectively. The above figures, while illustrating the enormous potential manurial wealth in the refuse of India, also show that the nitrogen-loss would be equivalent to no less than 150 lakhs of rupees (over one million pounds) on the finished product. The foregoing figures educe the importance of the organic manure industry and indicate the need for further efforts to minimise loss of nitrogen during preparation.

As exceptions to the above, heaps 1 and 15 show distinct nitrogen-gains of 16·5 and 8·5 per cent. respectively, thereby suggesting fixation of atmospheric nitrogen; both having been similar to the others except in treatment with activated sludge, the results indicate that fixation was due to that starter.

Evidence has been adduced by previous workers to suggest that fixation of atmospheric nitrogen may occur in manure heaps (*e.g.*, Tottingham, *J. Biol. Chem.*, 1916, **24**, 221). Richards noticed marked fixation during decomposition of horse-fæces in presence of calcium carbonate, and showed that the fixation was effected by a mixed culture of *azotobacter* and *B. lactis ærogenes* (*J. Agric. Sci.*, 1917, **8**, 299). Fowler observed that nitogen-fixing bacteria are present in activated sludge (*J. Indian Inst. Sci.*, 1921, **3**, 227), and noted that when biochemical starters were employed, there was positive increase in total nitrogen thereby suggesting fixation from the atmosphere (*Agric. J. India*, 1930, **25**, 363). Recently, Howard and Wad obtained similar evidence with respect to their Indore method (*loc. cit.*). In the light of the foregoing observations, it appears that under favourable conditions the original nitrogen can be retained and fresh quantities of that element be fixed from the atmosphere.

The technique of biological fixation has yet to be standardised: further work is needed to confirm the present observations on a more extensive scale with various types of raw materials, and to determine the various chemical and biological factors relating to fixation. These investigations are now in progress and will form the subjects of later communications.

Available potash and phosphoric acid.—These were estimated by the method of Dyer (*J. C. S.*, 1894, **65**, 115), and the results shown in Table V.

TABLE V.

Heap No.	Availability per cent. of K_2O	Availability per cent. of P_2O_5	Heap No.	Availability per cent. of K_2O	Availability per cent. of P_2O_5
1	0·09	0·27	9	0·08	0·34
2	0·10	0·26	10	0·11	0·22
3	0·09	0·27	11	0·08	0·28
4	0·07	0·24	12	0·13	0·25
5	0·10	0·22	13	0·06	0·24
6	0·05	0·23	14	0·09	0·27
7	0·04	0·20	15	0·11	0·29
8	0·10	0·27	16	0·06	0·33

All heaps contained moderate amounts of both constituents. No particular relation could, however, be traced between any of the treatments and the corresponding availability.

Influence of copper on the active microflora.—It has already been remarked that the decomposition of refuse proceeded at least as fast in heaps sprayed with copper-lime mixture as in those otherwise treated. In view of previous observations (Rege, *loc. cit.*, Norman, *loc. cit.*, Waksman and co-workers, *loc. cit.*) that decomposition of cellulosic materials is due to fungi, and the known fact that copper, even in minute quantities, is inimical to the growth and activity of such organisms, it was considered probable that the fungi present in the copper-treated heaps either became acclimatised to the presence of that element, or that the changes were wrought by other micro-organisms.

To verify the above, platings were carried out on different count media; mannite-asparagin agar for bacteria (*Ann. Appl. Biol.*, 1922, **9**, 241), starch agar for *actinomyces* (*J. Indian Inst. Sci.*, 1929, **12A**, 253) and Czapek's agar for fungi, with specimens of decomposing refuse from heaps treated with (*a*) raw sewage alone, (*b*) raw sewage with different chemicals, and (*c*) raw sewage with different chemicals but sprayed with copper-lime mixture. The observations showed that whereas fungi were the dominant flora in both *a* and *b* and persisted to a late stage in the decomposition, the reverse was the case in heaps treated with copper, where, within four weeks, the fungi diminished considerably while *actinomyces* increased almost six-fold. This suggests that the microflora concerned are profoundly affected by spraying with copper. The above observations are now being extended in the laboratory, not only with varying doses of copper, but also with other physiologically active inorganic compounds such as those of zinc and titanium.

Vegetation Experiments.

Pot-culture trials.—With a view to assessing the relative manurial values of the different preparations, pot-cultural trials were made with ragi (*Eleusine coracana*) during (1) autumn and winter of 1930, and (2) spring and summer of 1931. The pots were of ordinary earthenware and held about 40 lbs. of air-dry soil. They were made up with sand and soil in the usual way with 40 g. (each) of burnt lime, and after about a week were treated as follows:—(*a*) Sixteen sets with the finished products from the different heaps, at the rate of ½ lb. per pot and (*b*) one set each with activated sludge (62 g.), farmyard manure (1 lb.), and complete minerals ($NaNO_3$ 1 g., super 4 g., potassium sulphate 2 g.). One set of pots was also left untreated as control, and five pots were allotted for each treatment. Twelve seeds were sown per pot, and as the experiment progressed, representative plants were collected from each pot, for thinning and to give sufficient material for growth measurements and chemical analyses. From several sets of determinations the following figures obtained at three different stages during one season (1931) will illustrate the relative values (Table VI).

Table VI.

Manure from Heap No.	Root-length in cm.			Shoot-height in cm.			Root-weight per plant in g.			Shoot-weight per plant in g.		
	DAYS			DAYS			DAYS			DAYS		
	21	38	52	21	38	52	21	38	52	21	38	52
1	22	35	44	12	48	56	0.04	0.46	1.71	..	0.56	1.92
2	25	40	43	14	47	60	0.04	0.66	1.08	0.07	0.58	1.67
3	22	36	45	11	44	56	0.03	0.41	1.04	0.04	0.60	1.47
4	22	30	55	18	47	55	0.04	0.45	1.44	0.05	0.78	1.06
5	24	27	46	18	50	50	0.06	0.41	1.48	0.01	0.67	1.14
6	21	47	50	14	45	65	0.04	0.84	1.47	0.07	0.61	1.22
7	22	24	27	11	40	40	0.03	0.27	0.55	0.07	0.46	0.46
8	24	28	45	12	43	54	0.05	0.47	1.21	0.06	0.47	1.23
9	24	29	44	15	42	67	0.04	0.51	0.95	0.09	0.82	1.85
10	20	29	44	13	36	46	0.03	0.37	0.92	0.06	0.39	0.57
11	24	29	30	15	39	53	0.07	0.23	0.56	0.02	0.33	0.92
12	24	30	38	14	44	56	0.03	0.50	0.80	0.04	0.63	1.60
13	22	26	46	12	40	54	0.04	0.25	1.17	0.03	0.43	1.09
14	19	40	..	11	38	48	..	0.26	0.44	..	0.40	0.75
15	17	29	47	14	39	61	0.04	0.37	1.11	0.05	0.60	1.75
16	26	37	48	15	46	52	0.05	0.44	0.88	0.06	0.78	0.65
Activated sludge (dry powder) ..	22	33	44	17	53	73	0.05	0.72	1.95	0.14	1.56	3.38
Farmyard-manure ..	21	35	44	16	47	73	0.02	0.64	0.75	0.12	0.51	1.25
Complete minerals	19	33	47	7	40	48	0.01	0.29	0.78	..	0.13	0.12
No Manure ..	12	×	×	3	×	×	0.003	×	×	0.002	×	×

.. Data not available. × Plants made poor growth and died prematurely.

No relation could be traced between the root-length or shoot-height and any of the treatments, nor does the root-weight throw any light on plant development. The shoot-weight being that of the portion above ground is a useful index of growth, and being composed largely of those of the tillers, it may also be considered as the index of relative tillering capacities of the plants under various treatments.

The results show that although they are inferior to activated sludge, many of the refuse-manures, particularly those sprayed with raw sewage, are slightly superior to farmyard manure and even more superior to combined chemical fertilisers.

Plot experiments.—The crop yields under field conditions were compared by the foregoing experiments repeated on plots, 10 ft. by 10 ft. and separated by raised ridges of about 1 foot width. Three were allotted for each manurial treatment, and to minimise inherent errors due to soil and other variations, were distributed at random (Fisher, Statistical Methods for Research Workers, 1925). All the plots were first treated with lime at the rate of 2 tons per acre, and after about a fortnight were divided into four groups as mentioned above and treated with (*a*) farmyard manure at 25 tons to the acre, (*b*) organic manure obtained by spraying raw sewage on town refuse on a nitrogen basis equivalent to *a*, (*c*) complete minerals consisting of (1) nitrate of soda at 80 lbs., (2) superphosphate (concentrated) at 4 cwts, and (3) potassium sulphate at

2 cwts. per acre, and (*d*) no manure as control. Ragi seedlings about a month old were transplanted into the plots, and when the crops came to harvest after about three months were removed, and the yields of grain and straw determined (Table VII).

TABLE VII.

Treatment	Yields: Ears lbs.	oz.	Grain lbs.	oz.	Straw lbs.	oz.	Grain / (Grain + Straw)
Refuse-manure ..	24	0	8	8	187	8	0·043
Farmyard-manure ..	23	0	8	2	109	2	0·069
Chemical fertilisers ..	21	2	6	6	89	6	0·069
No manure ..	14	2	5	0	76	2	0·062

It may be seen from the above that the refuse-manure is slightly superior to farmyard-manure when applied on an equivalent nitrogen basis. The results compare more favourably with those for chemically fertilised or unmanured plots. The yield of straw from the refuse-manured plots is disproportionately higher than that of grain when compared with those from other plots. The observations suggest either excessive supply of available nitrogen or absence of certain essential manure constituents that would have helped to increase the proportion of grain to straw. Later trials showed, however, that the effect was at least partly due to insufficiency of available phosphorus: when superphosphate was first applied as a basal dressing to the soil, the proportion of grain to straw showed a distinct increase, thus resulting in a higher yield of grain than that recorded in the above table.

Residual value of the refuse-manure.—After removing the first crop the soil was allowed to rest for three months, then ploughed and sown with ragi seedlings as in the previous experiment; the crops were harvested and the yields of grain and straw determined (Table VIII).

TABLE VIII.

Previous treatment	Yield: Grain lbs.	oz.	Straw lbs.	oz.
Refuse-manure	2	15	17	0
Farmyard-manure	1	12	14	3
Chemical fertilisers	0	13	9	14
No manure	1	7	9	0

The results show that the residual value of the refuse-manure is higher than that of the others; the effect would probably have been still more marked if the soils had been given a longer period of rest, as is usual in dry farming practice. Comparison of yields from plots treated with chemical fertilisers and without manure shows that although the plants were of about the same weight (as indicated by the combined weights of straw and grain) in both the cases, the yield of grain was very much less in the former than in the latter. This observation is rather peculiar and further work is needed to elucidate its significance in tropical farm practice.

Copper toxicity to plant life.—It being possible that the copper mixture in some heaps is toxic to plant growth (Brenchley, Inorganic Plant Poisons and Stimulants, 1927), aqueous and saline extracts of specimens from such heaps were prepared and tested for the presence of copper with negative results. This in conjunction with the pot and plot experiments clearly shows that copper as present in the heaps is non-leachable in appreciable quantities, and will not therefore have any deleterious action on plant growth. It should be noted at the same time that the total quantity of copper in the finished manure, even after repeated sprayings, is very small and could not therefore be expected to appear in appreciable quantities in the extracts. Thus, a heap weighing 1000 lbs. would contain only about 25 g. of copper sulphate (0·006 per cent. on the dry weight), and being applied with a considerably larger proportion of lime, all of it may be expected to have turned rapidly into insoluble carbonate.

It is difficult to state whether appreciable quantities of copper would not pass into solution in presence of the organic acids formed under the swamp soil conditions (Subrahmanyan, *J. Agric. Sci.*, 1929, **19**, 627) when the refuse-manure is applied to paddy fields; but even under such conditions, it is probable that the minute quantities released may actually prove beneficial to plant growth. Field observations in America have shown that increased yields can be obtained by giving top dressings of copper sulphate at 50 lbs. per acre (*Florida Agric. Expt. Sta., Bull.* 190, 1927). Quartaroli (*Ann. Chem. Applicata*, 1929, **19**, 467) has adduced evidence to show that copper is ten times as efficient as manganese in promoting plant growth, and has indicated that copper is related to plant life as are vitamins to animal nutrition. Similar observations on the importance of copper to plant growth are due also to Sommer (*Plant Physiology*, 1931, **6**, 339).

Cost of making the refuse-manure.—Both refuse and sewage are waste materials which have to be removed from populated areas and it follows that whether converted into manure or not, the refuse must be collected and transported. The cost of making the refuse-manure should therefore normally be only that of labour, and machinery for treating the refuse with sewage, chemicals or other materials to facilitate decomposition at the place of treatment. Observations on the experimental heaps have already shown that addition of chemical starters is unnecessary and indeed wasteful. The cost of making the manure therefore reduces itself to that of (*a*) spraying with sewage, and (*b*) treating with insecticides, fungicides or other substances to eliminate odour and suppress putrefactive and pathogenic organisms.

Assuming that the heaps are arranged in a row or a continuous chain and that coolies provided only with buckets and watering cans are available, it may be expected that each cooly can maintain at least 40 tons of refuse sufficiently moist for continuous decomposition. Our observations have shown that the decomposition is complete in about two months, but allowing a margin of a fortnight and reckoning that the cooly is paid 8 As. (9*d.*) a day, it may be calculated the total average cost of spraying a ton of refuse would be

15 As. The cost of copper sulphate required for spraying one ton of refuse would be less than half an anna and that of lime about one anna. The total cost of treatment would therefore be about one rupee (18*d.*) per ton of refuse. There would no doubt be shrinkage in dry matter to the extent of about 20 per cent., but the finished product generally contains an equivalent amount of extra moisture so that the final weight of the manure would be about the same as that of initial refuse. It may be reckoned therefore that the cost price of the manure would be about one rupee per ton.

The composition and the price paid for farmyard-manure vary from place to place, and are determined by several factors such as the number and feed of farm animals or local demand. It may be assumed, however, that the average type of farmyard-manure available in India contains about 0·7 per cent. nitrogen and fetches about Rs. 5 per ton, whence it follows that the refuse-manure which is rich in nitrogen (about 1 per cent.) and has been shown to possess a greater manurial value would be worth more than Rs. 5 a ton. Since refuse-manure can be made at about Re. 1 per ton, it can be sold at less than half the price of farmyard-manure and still show ample profit.

In large cities where the volume of refuse is considerable and where power is easily available, it would be cheaper and indeed more efficient to spray the sewage by machinery. Devices for spraying are already known and adopted in sewage practice, so that with the introduction of the necessary equipment large volumes of sewage and refuse can be dealt with at far less cost than when employing man-power alone. In European countries, particularly Great Britain, where farmyard-manure costs 4-5 times as much as it does in India and where machine-power is cheaply available, it would be highly profitable to convert all the available refuse into manure, and so meet the growing demand from those engaged in glass-house industries, market-gardening and fruit-farming.

Experiments in composting refuse with night-soil.—The problems connected with the utilisation of night-soil in India resemble those in China and Japan, night-soil being removed by a system of hand conservancy and subsequently distributed: in China and Japan after decomposition in special cisterns or underground pits, and in India generally by direct application to land. Both systems are unsatisfactory hygienically. The Chinese method though preferable agriculturally is insanitary from smell and water-pollution. The Indian method is also defective, but the night-soil being merely heaped on the fields offers less danger of water-pollution than in China. Moreover, the use of unmixed night-soil leads to considerable wastage of nitrogen, owing to its high proportion of nitrogen to carbon.

Fowler (*Surveyor*, 1927, **71**, 259) suggested that night-soil may be dumped on refuse heaps and the mixture turned frequently so as to facilitate ærobic decomposition. In a later publication (*Agric. Jour. India.*, 1930, **25**, 363), he described experiments at Nasik where night-soil was used both as the source of nitrogen and as the requisite biological activator, obtaining a well-rotted manure in a few weeks.

Some preliminary experiments were conducted to determine the effect of adding night-soil in small instalments to refuse heaps, one gallon per heap (500 lbs.) of refuse; the heaps were then turned and moistened with raw sewage at the rate of 20 gals. per heap. It was found difficult, however, thoroughly to mix the night-soil with the heap, and after about 25 days the difficulty increased, unpleasant odours being emitted when the heaps were stirred. Spraying with copper-lime mixture or bleaching powder suppressed the smell, but only temporarily. It was easier and more hygienic to suspend the night-soil in sewage and then distribute the liquid over the heap.

Night-soil (2 gals.) was stirred into 12 gals. of raw sewage in a covered drum of about 25 gals. capacity. After 10 days the contents were homogeneous and in spite of some odour could be handled with ease. A portion was transferred to another drum, mixed with 10 times its volume of raw sewage, and then applied to the refuse heaps. To the residue in the first drum, more night-soil was added and liquefaction allowed to proceed as before, being complete within 24 hours; a portion was removed and applied to the refuse heaps after dilution with raw sewage in the manner described already. The processes of liquefaction, dilution and spraying were repeated from day to day without experiencing any further difficulty.

The sprayed heaps were (*a*) entirely of refuse and (*b*) half refuse, and half mixed waste vegetation, mostly lantana (*Lantana camara*) leaves. The heaps of refuse alone weighed about 240 lbs. each while those mixed with waste vegetation weighed about 480 lbs. each and were all treated with lime corresponding to 1 per cent. on the dry weight. One heap weighing 240 lbs. was used as control and sprayed only with 18 gals. of water per day. Heaps under *a* were sprayed with night-soil emulsion (prepared in the manner described above) at the rate of 6 gals. per day and those under *b* at double that rate. It was soon found, however, that the above-mentioned quantities did not provide sufficient moisture to facilitate rapid decomposition, and therefore 12 and 24 gals. respectively of raw sewage per day were used. Each heap was stirred twice a day and sprayed with minute quantities of copper-lime mixture or bleaching powder suspension whenever there was a pronounced smell; but the insecticides and fungicides were needed only occasionally, and there was normally no foul odour from any of the heaps. The contents of the drum used for liquefying night-soil were somewhat objectionable particularly after fresh additions.

The foregoing observations, though essentially preliminary in character, have brought out important facts relating to the utilisation of night-soil with sewage in composting refuse. Preliminary liquefaction of night-soil is essential to its adequate admixture with the refuse, and although initially the active liquefying flora are slow to establish themselves, subsequent liquefaction proceeds at a rapid rate thus facilitating the utilisation of liquefied night-soil within the 24 hours after collection; but the technique of dilution and application requires further improvement.

It was observed that the liquefied and diluted night-soil as applied to the refuse heaps contained on an average 52 parts of nitrogen per 100,000. This was very considerable as compared with raw sewage, and it was expected that the final product would be richer in nitrogen than the heaps sprayed with raw sewage alone. The decomposition of the heaps treated with night-soil emulsion was allowed to proceed for two months when appreciable change in dry matter had ceased; the products were removed, dried and analysed for the various fertilising ingredients, the percentage composition of the different preparations on the oven-dry basis being given in Table IX.

TABLE IX.

Manure from	*Nitrogen* Total	Ammonia	Nitrate	Albu-minoid	*Availability* P_2O_5	K_2O
Refuse+waste vegetation*	0·90	0·03	0·22	0·35	2·54	0·27
Refuse only*	0·92	0·02	0·15	0·32	0·60	0·37
Refuse sprayed with water only	0·46	0·01	0·11	0·21	0·33	0·13
Farmyard-manure	0·72	0·02	0·11	0·27	0·30	0·40

* Treated with night-soil emulsion and sprayed with sewage.

It is seen that although the manure prepared by composting refuse with night-soil is richer in various fertilising ingredients than farmyard-manure, it is not greatly superior to most of those previously obtained by spraying raw sewage alone on refuse. The product obtained from the mixture of refuse and waste vegetation is, however, distinguished from the others by its richness in available phosphoric acid, which it contains to the extent of over four times that of any of the heaps so far examined. Since those heaps were identical with the others except in that they were half waste vegetation it may be inferred that the increased phosphorus content was derived from that component.

A study of the nitrogen balance shows that the loss of nitrogen was more marked in the case of heaps treated with night-soil emulsion than in those sprayed with raw sewage alone. The results of the present experiment indicate that the loss corresponds to about 23 lbs. of nitrogen per ton of finished product. This suggests that in addition to standardising conditions for the application of night-soil emulsion to the refuse attempts should also be made to minimise the loss of nitrogen occurring under such conditions.

The foregoing observations also show that when supply of refuse is inadequate for being composted with the available amount of night-soil it can be advantageously mixed with waste vegetation so as to obtain a suitable finished product. As the chemical composition of the waste vegetation used for the experiments had not been previously determined, it is difficult to state whether the loss of nitrogen was greater in their presence than in refuse alone. Further work is needed to elucidate the above and related problems.

Vegetation experiments with manure prepared from night-soil and refuse.—To compare the relative manurial values of (1) manure prepared by composting refuse with night-soil, (2) farmyard-manure, and (3) the product obtained by spraying refuse with water, pot-culture experiments were made with ragi in the manner described already. Growth measurements were taken at various stages and Table X shows the results obtained on the 52nd day after sowing.

TABLE X.

Manure	Root-length in cms.	Shoot-height in cms.	Root-wt. in g.	Shoot-wt. in g.
Refuse (night-soil)	57	57·5	0·97	2·40
Farmyard-manure	54	46·5	0·47	1·80
Refuse (water)	56·5	43·5	0·40	0·97

As distinguished from the observations made in the earlier set of experiments, it was also noted that the plants raised on refuse (night-soil) manure had a larger root-system in addition to having bigger and richer tops. Since it is known that phosphates are generally helpful to root development and since the night-soil treated manure is richer in phosphorus than the preparation sprayed with sewage, it is possible that the increased root development was due to a larger amount of that element in the former.

Plot experiments.—Field experiments were made with (1) night-soil treated manure and (2) farmyard-manure, to compare their relative manurial values and to determine the efficacy of giving basal dressing of superphosphate on crop yield. The areas of the plots, and their distribution were identical with those described in an earlier experiment. The total area under each treatment was 300 sq. ft. and the two manures were added on equivalent nitrogen basis, farmyard-manure being taken as the standard and applied at 25 tons to the acre. After allowing the soil to rest for a week, superphosphate (concentrated) was added at 4 cwts. to the acre and after a further rest, the plots were sown with ragi seedlings as in the previous trials. After about three months, the crop was harvested and the yields of grain and straw determined.

TABLE XI.

Total Yields.

Manure	Straw		Grain	
	lbs.	oz.	lbs.	oz.
Refuse-manure ..	41	8	12	14
Farmyard-manure ..	24	8	8	0

The above results bear testimony to (*a*) the distinct superiority of the refuse-manure to farmyard-manure and (*b*) the efficacy of superphosphate applied in combination with the refuse-manure in increasing crop yield. It should, however, be noted that the rates at which the manures were applied, in the above and the previous trials were larger than those commonly adopted in Indian agricultural practice. Further experiments should be carried out to

determine the optimum dosage of the phosphate as well as the organic component.

Experiments on composting with activated sludge.—Although the manurial value of activated sludge has long been recognised, its application in agricultural practice has been greatly handicapped by difficulties connected with de-watering. Fresh sludge contains nearly 98 per cent. of water and retains it tenaciously unless treated with acids, electrolytes or heat. Applying the above principles, much work has been done by various workers in America (Martin, Activated Sludge Process, 1927, 326-347) and by Fowler in India. The cost of de-watering appears to be rather high compared with the sale-value of the fertiliser. In view of the above and other difficulties attending the de-watering of sludge, several workers have preferred the digestion process; this yields fuel gases and an inoffensive, tarry residue, but is wasteful and results in loss of valuable fertilising ingredients.

Consequently, since refuse heaps readily abstract water from sewage sludges, fresh activated sludge (2 litres) was added to refuse (8 lbs.) and the daily changes noted. Control experiments were also carried out by leaving similar lots of activated sludge to undergo spontaneous evaporation in shallow containers. It was observed that whereas the treated refuse absorbed all the sludge and underwent rapid decomposition accompanied by development of an earthy odour and steady loss of moisture, the sludge left in containers soon underwent septic action accompanied by foul odours but no marked loss of moisture. Determinations of moisture content of treated refuse at intervals gave the following results:—

Day	Moisture per cent.	Day	Moisture per cent.
1	73·9	10	25·0
5	55·2	12	13·4
6	35·8	14	11·1

The results sh[illegible] that although the dehydration is not immediate, it proceeds steadily, and the cost would be very small. The final product would have increased manurial value from the fertilising ingredients of both activated sludge and refuse.

Summary.

In presence of mineral starters neither the rate of decomposition nor the manurial value of refuse composted with sewage is appreciably improved.

Owing to inadequate æration and other reasons the heaps tend to smell occasionally, but normal conditions may be rapidly restored by spraying the heaps with either thin Bordeaux mixture or bleaching powder suspension, the former being cheaper and more efficient.

Under normal conditions, there is marked loss of nitrogen from the decomposing heaps approximating to 2 lbs. on the preparation of each ton of manure. In presence of activated sludge, however, there is an increase in total nitrogen suggesting fixation of that element from the atmosphere.

Vegetation experiments have shown that the organic manures prepared from refuse are generally superior to farmyard-manure and more effective than combined chemical fertilisers. As in most cases, the value of the manure appears to be enhanced by superphosphate. The residual value of the refuse-manure is also higher than that of farmyard-manure and other fertilisers tested.

The possibilities of the refuse-manure industry have been discussed. Even in absence of machine-power for spraying the sewage, the cost of making the manure is about one-fifth of the price of comparatively inferior farmyard-manure. The cost could be further reduced by employing machine-power and by so adjusting the proportion of sewage or night-soil to refuse and ensuring adequate air-supply that the need for spraying with chemicals to suppress odour becomes unnecessary. The supply of raw material is enormous, the annual collection of refuse being over 20 million tons in India alone. It could also be supplemented by combining the refuse with waste vegetation.

Marked change in the composition of the microflora concerned in the decomposition is brought about by spraying the heaps with the copper mixture. *Actinomyces* are greatly encouraged while fungi are suppressed. The change in the microflora does not, however, affect either the rate of decomposition or the manurial value of the final product.

Experiments on composting with night-soil have shown that it would be necessary partially to liquefy the material and then dilute it with sewage to ensure adequate distribution over the heaps. With proper æration, the decomposition proceeds rapidly, but the loss of nitrogen is greater than in the case of heaps treated with sewage alone.

By mixing activated sludge with refuse it may be possible to reduce the water-content of the former and at the same time facilitate the decomposition of the latter.

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I.—STUDIES IN ENZYME ACTION—PART V.
AGEING OF AMYLASES IN AQUEOUS SOLUTION.

By K. Venkata Giri and V. Subrahmanyan.

Although it is well known that preparations of enzymes lose their activity under a variety of conditions, the processes leading to such inactivation have not, so far, been properly understood. Age, light and other radiations, prolonged dialysis, mechanical agitation, accumulation of reaction products, and poisonous chemicals lead to marked fall in activity resulting in complete inactivation. The adverse effects due to most of the above can be prevented by proper control of conditions, but no suitable method has, so far, been devised to thwart the effect of age with the result that large amounts of originally valuable preparations have to be rejected from time to time, thereby involving manufacturer and consumer in considerable loss.

Sherman and Schlesinger (*J. Amer. Chem. Soc.*, 1911, **33**, 1195; 1912, **34**, 1100) observed that solutions of pancreatic amylase undergo rapid deterioration on keeping. Fenger and Hull (*J. Biol. Chem.*, 1921, **46**, 431) noted that all pancreatic enzymes excepting trypsin become inactivated on storage. Sumner and Hand (*J. Biol. Chem.*, 1928, **76**, 149) observed that urease is readily inactivated to the extent of 80 per cent. in aqueous solutions.

Sherman and Schlesinger (*loc. cit.*) attributed the inactivation of pancreatic amylase to hydrolysis of the enzyme by the associated proteases. This view is not, however, supported by the observation of Willstätter and his co-workers (*Z. Physiol. Chem.*, 1926, **126**, 143) who find no evidence for the presence of protein in their purified preparations. Abderhalden and Guggenheim (*Z. Physiol. Chem.*, 1910, **68**, 317) and other workers, who noted that certain enzyme preparations are rapidly inactivated on vigorous shaking, attributed their observation to certain physical changes undergone by the enzymes. Working with crystalline pepsin, Northrop (*J. Gen. Physiol.*, 1930, **13**, 740) observed that the inactivation of the enzyme by heat or alkali is quantitatively proportional to the denaturation of the enzyme protein. Falk (*J. Biol. Chem.*, 1917, **31**, 97) who studied the inactivation of lipolytic enzymes found no evidence of protein hydrolysis, but obtained indications to show that under such conditions the enzymes undergo tautomeric changes. Sumner and Hand (*loc. cit.*), on the other hand, found that the rapid inactivation of aqueous solutions of crystalline urease was due to traces of lead present in the distilled water used by them.

The foregoing observations show that the mechanism of enzyme inactivation under different conditions has not yet been fully understood; an investigation was therefore undertaken to throw further light on the problem. The present paper deals with the changes that accompany the ageing of amylases in aqueous solutions.

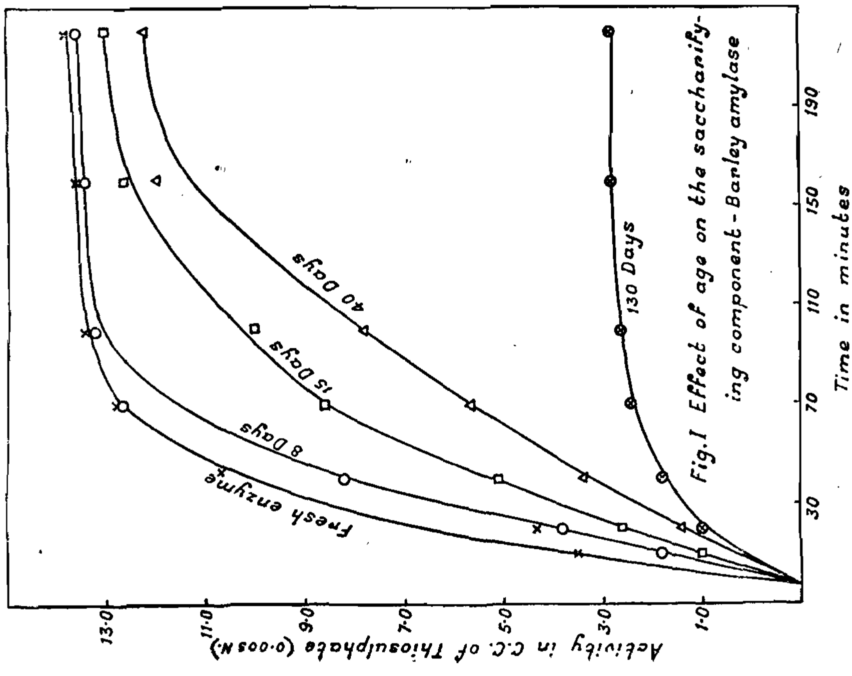

Fig. I Effect of age on the saccharifying component - Barley amylase

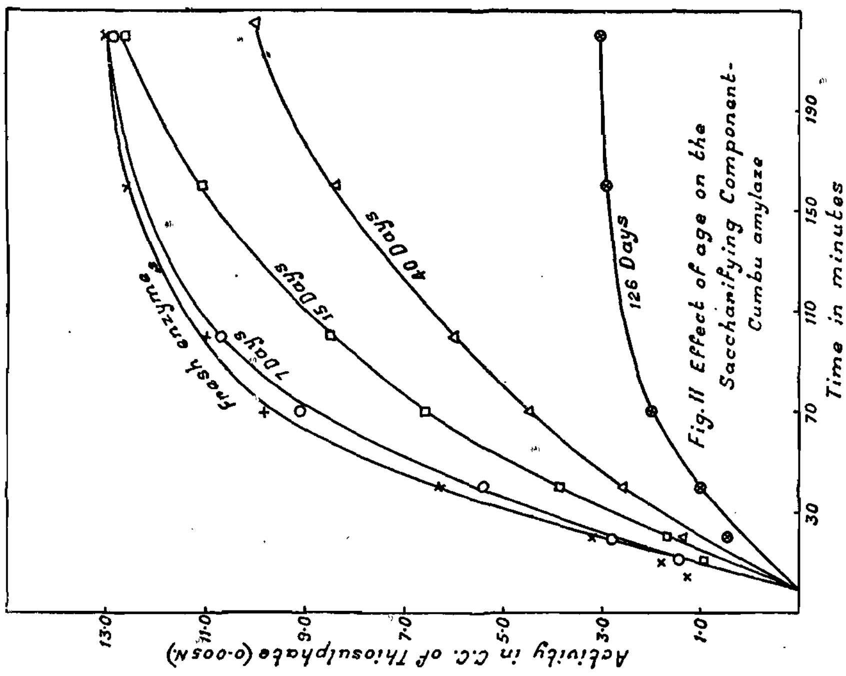

Fig. II Effect of age on the Saccharifying Component - Cumbu amylase

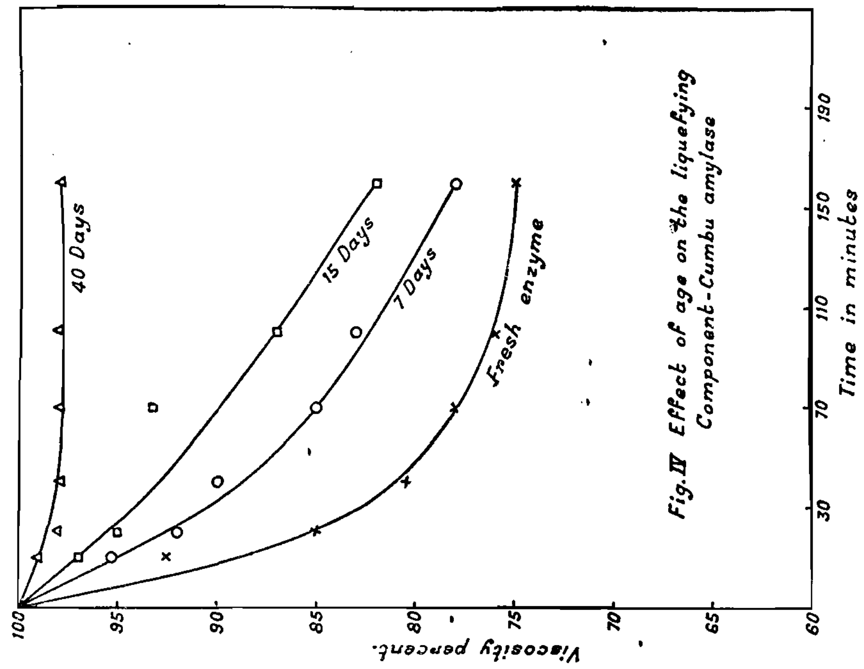

Fig. II Effect of age on the liquefying Component-Cumbu amylase

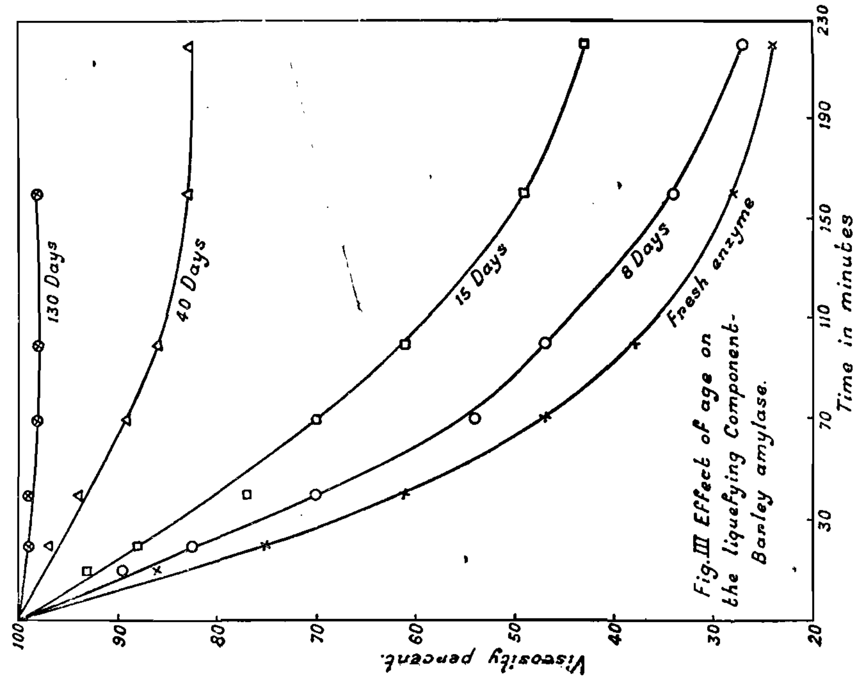

Fig. III Effect of age on the liquefying Component-Barley amylase.

Experimental.

The preparations of amylases used in the present study were those derived from barley and cumbu (*Pennisetum typhoideum*). Some experiments were also conducted with Taka-diastase (Parke-Davis).

Preparation of Enzymes.—The grains were steeped in running water for 36–48 hours and then spread out to germinate on moist sand for about 3 days at 27–29°. The germination was then arrested, the grains freed from sand and dried first in the sun and then under reduced pressure at 40° for 4–6 hours. The dried plumules were then removed by light crushing and the malts powdered.

The extracts were prepared by treating 400 g. of powdered malt-meal with 1200 c.c. of distilled water in presence of toluene for 3 days at 27–29°. They were then filtered through paper pulp and dialysed in collodion bags against distilled water for a week, when all the proteins were precipitated and the sugar removed. They were then centrifuged to remove suspended matter, the resulting clear liquid being treated with toluene and maintained at constant temperature (27–29°) in a dark chamber and free from dust.

Effect of age on saccharifying component.—Previous work (particularly, Fricke and Kaja, *Ber.*, 1924, **57**, 310, 313; Narayanamurti and Norris, *J. Indian Inst. Sci.*, 1928, **11A**, 134) having established that vegetable amylases are made up of liquefying as well as saccharifying components, experiments were made to determine the relative effects of ageing on the two components.

The changes in saccharogenic power were determined in the following manner:—The reaction mixture containing 40 c.c. of starch solution (2 per cent.), 10 c.c. of Walpole's acetate buffer at the optimum P_H for the enzyme, 5 c.c. of enzyme solution and 25 c.c. of water with a few crystals of thymol as antiseptic, was maintained at 34°. Aliquots were removed at intervals and the sugar-content determined by the method of Schaffer and Hartmann (*J. Biol. Chem.*, 1920–21, **45**, 365), the results being assembled in Figs. I and II. Although both the preparations lost the major part of their activity at the end of 130 days, the rate of inactivation was slower in the case of barley amylase than in that of the cumbu enzyme. In the latter case, there was a distinct fall in activity even at the end of 40 days.

Effect of age on liquefying component.—This was determined by the viscosimeter method under conditions similar to those adopted in the previous experiment; the results have been plotted in Figs. III and IV. There was distinct fall in the liquefying power even at the end of 40 days. The effect was more marked in the case of the cumbu enzyme than in that of barley. In both the cases the liquefying power was almost entirely lost at the end of 130 days.

A comparison of the above with the previous observations shows that the liquefying power is more readily lost than that of saccharification, thereby suggesting that the saccharifying component is less affected by age than the liquefying one under the conditions of the present investigation.

Change in optimum P_H.—Experiments were conducted with solutions of Taka-diastase (0·2 per cent.) in addition to those with barley and cumbu enzymes to determine whether the optimum P_H of the enzymes is affected as the result of ageing. Reaction mixtures composed of 10 c.c. of soluble starch (2 per cent.), 10 c.c. of Walpole's acetate buffer adjusted to varying P_H and 5 c.c. of enzyme at each stage of ageing were maintained at constant temperature and the activities determined at the end of half an hour. In the case of malt amylases the temperature of reaction was 35° while with Taka-diastase it was 40°: in the former, sugar was estimated by the method of Schaffer and Hartmann (*loc. cit.*) and in the latter gravimetrically, by the cuprous oxide method. The results have been given in Tables I*a*, I*b* and I*c*.

TABLE I *a*.

Change in Optimum P_H *—Barley Amylase.*

P_H	Activity in terms of c.c. of Thiosulphate (0.005*N*)				
	Fresh enzyme (control)	Age of enzyme in days			
		7	15	40	130
6·2	6·4	4·9	4·1	3·5	0·4
5·9	8·4	..	5·3	3·5	0·9
5·2	8·6	8·0	6·7	5·6	*1·7*
5·1	8·9	9·5	6·8	*6·0*	*1·7*
5·0	9·2	9·7	*7·0*	5·8	1·6
4·8	11·0	*9·9*	6·8	5·8	1·6
4·6	*11·4*	9·7	6·6	5·5	1·6
4·4	11·2	9·3	6·4	5·4	..
4·2	10·6	9·0	..	5·2	1·5
4·0	10·3	8·5	6·2	5·2	1·45
3·0	8·5	8·5	6·0	5·2	1·5

TABLE I *b*.

Change in Optimum P_H *—Cumbu Amylase.*

P_H	Activity in c.c. of Thiosulphate (0.005*N*)				
	Fresh enzyme (control)	Age of enzyme in days			
		7	15	40	126
6·0	7·0	6·0	4·0	1·0	0·6
5·8	7·2	6·8	4·4	2·0	1·6
5·5	7·2	7·2	5·0	3·4	*2·4*
5·3	7·6	7·4	*6·6*	*4·4*	2·2
5·2	8·6	8·0	5·6	4·0	2·2
5·1	8·6	*8·4*	5·2	3·8	2·2
5·0	*10·0*	7·8	5·4	3·8	2·2
4·8	8·8	7·4	5·8	3·6	2·4
4·6	8·0	7·4	6·4	..	2·2
4·4	8·0	7·2	6·4	3·2	2·4
4·0	8·8	7·4	6·0	3·4	..
3·7	8·0	7·2	5·6	3·4	2·4

TABLE I *c*.

Change in Optimum P_H—*Taka-diastase.*

P_H	Activity in terms of mg. of Cu_2O		
	Fresh enzyme (control)	10 days	25 days
6·2	271·6	235·2	205·0
5·4	323·6	274·8	240·0
5·2	328·2	279·0	240·0
5·0	*332·6*	*281·2*	*240·0*
4·8	323·6	276·6	238·6
4·6	319·8	272·4	233·0
4·3	295·4	254·6	219·2
3·6	209·6	181·2	158·8
3·1	154·2	135·6	..
Without buffer	285·8	237·6	207·4

It may be noted that (1) the optimum P_H in the two malt amylases is changed to a less acid one, and (2) the zone of optimum P_H tends to become *wider as the result of ageing. There is no perceptible shift of optimum* P_H *in* the case of Taka-diastase though, as in the case of the other enzymes, the zone becomes wider with ageing.

Similar changes in the optimum P_H of enzyme preparations have been recorded by Nelson and Bloomfield (*J. Amer. Chem. Soc.*, 1924, **46**, 1025) who studied the effect of temperature on invertase, and by Sherman and co-workers (*J. Amer. Chem. Soc.*, 1928, **50**, 2529) who investigated the effect of neutral salts on pancreatic amylase. The evidence so far obtained is not sufficient to explain the shift in optimum reaction in the case of the malt amylases, but in view of the previous observations it appears that the saccharifying enzyme, which is the only component left in the later stages, has a wide range of optimum reaction, particularly on the acid side.

Stability at different H-*ion Concentrations.*—Solutions of the two malt amylases were dialysed against distilled water for a week. They were then made up to constant volume and aliquots (50 c.c.) transferred to bottles each containing 20 c.c. of Walpole's acetate buffer of a particular reaction. After adding toluene, the mixtures were maintained at 27–29° and the activities determined, from time to time, after adjusting the final reaction, in each case, to the optimum P_H of the enzyme concerned. Control experiments were also conducted with enzyme solutions to which buffers had not been added. The results have been presented in Tables II *a* and II *b*.

TABLE II *a*.

Stability of Cumbu Amylase at different H-ion Concentrations.

P_H	Activity in c.c. of Thiosulphate (0.005N)			
		Age in days		
	Fresh enzyme	7	22	40
Without buffer	5·3	5·0	2·7	1·8
6·50	5·4	5·0	2·7	1·3
5·57	5·3	4·9	2·6	1·3
4·99	5·4	4·9	3·2	2·1
4·60	5·2	5·0	3·5	2·6
3·70	5·3	5·0	2·7	1·3
2·69	5·4	5·1	2·5	1·0
Undialysed enzyme	12·6	12·4	11·8	11·0

TABLE II *b*.

Stability of Barley Amylase at different H-ion Concentrations.

P_H	Activity in c.c. of Thiosulphate (0.005N)		
		Age in days	
	Fresh enzyme	15	40
Without buffer	17·0	15·8	10·9
6·50	17·1	15·5	10·0
5·57	17·0	15·7	10·2
4·99	17·1	16·1	11·0
4·62	17·1	16·2	11·0
3·72	17·0	*nil*	*nil*
2·69	17·0	*nil*	*nil*

It may be noted that all the dialysed preparations lost their activity more readily than the undialysed ones though the reactions were subsequently maintained constant by addition of buffers. The observations suggest that during dialysis certain components essential for the stabilisation of the enzymes are lost. In the case of the buffered preparations, inactivation proceeded more slowly in the neighbourhood of the optimum reaction (P_H 4·6) than at others. The barley enzyme was more sensitive to reactions on the acid side of the optimum P_H than the cumbu preparation.

A similar experiment with undialysed Taka-diastase (0·2 per cent.) gave the results in Table III showing that the enzyme is most stable at P_H 6·2 which is less acid than the optimum (P_H 5·0) for the enzyme. The enzyme deteriorates readily as the medium becomes more acid.

TABLE III.

Stability of Taka-diastase at different H–ion Concentrations.

P_H	Activity in mg. of Cu_2O			
	Fresh enzyme (control)	Age of enzyme in days 8	19	50
6·2	*333·4*	*309·0*	*302·6*	*292·6*
5·6	,,	299·0	269·8	236·2
5·1	,,	253·8	209·4	142·4
4·2	,,	39·8	29·8	27·6
3·4	,,	28·0	26·2	24·0
2·7	,,	25·6	25·0	20·2
Unbuffered	,,	285·2	220·6	140·4

The relation of the stabilising reaction of an enzyme to its optimum P_H is comparatively obscure. Although many of the commoner enzyme preparations resist heat inactivation more effectively at their optimum P_H than at any other reaction, cases of exceptions are also known. Thus Northrop (*J. Gen. Physiol.*, 1922, **4**, 261) observed that trypsin is most resistant to heat at P_H 6·0 though its optimum reaction is P_H 8·0–10·0. Ege (*Z. Physiol. Chem.*, 1925, **143**, 159) noted that pepsin is most stable at P_H 3·6–4·6 though its optimum reaction is P_H 1·6.

Effect of concentration on ageing.—Solutions of cumbu amylase of two different concentrations (A and B) were dialysed, treated with toluene and their activities determined, suggesting that the enzyme solution is more stable concentrated than when dilute (Table IV).

TABLE IV.

No. of days	Activity in c.c. of thiosulphate A	B
0	16·3	9·2
10	15·7	8·0
40	9·2	3·5

Changes in the P_H of the enzyme solution.—Solutions of barley and cumbu amylases were dialysed, treated with toluene and maintained at 27–29°. Changes in P_H were determined colorimetrically at intervals (Table V).

TABLE V.

P_H

Source of Amylase	Before dialysis	After dialysis—days 0	7	20	40
Barley	6·0	6·1	5·4	..	4·6
Cumbu	5·9	6·2	5·5	4·4	4·2

The results show that the reaction of the enzyme solution tends to become more acid on keeping: a similar experiment conducted with Taka-diastase showed that the reaction of the solution changed, in a like manner, from P_H 6·2 to 5·2. This might have been due to either production of fresh acid or removal of buffering materials originally present in the enzyme solution: further work is necessary to elucidate the point, but the observations of Graber (*Indus. Eng. Chem.*, 1914, **6**, 403) who detected lactic acid in glycerine extracts of malt. are rather suggestive in this connection.

Attempts to reactivate aged enzymes.—The observations of Onodera (*Biochem. J.*, 1915, **9**, 544) and others having shown that the activities of enzyme preparation can be restored under certain conditions, some experiments were conducted to ascertain whether inactive amylases can be revived under similar conditions.

To determine whether fresh or boiled enzymes could provide the necessary activators, mixtures of the following compositions were prepared and their activities determined:—(1) Boiled enzyme+water, (2) boiled enzyme+aged enzyme, (3) aged enzyme+fresh enzyme, (4) aged enzyme+water, and (5) fresh enzyme+water. Equal volumes (10 c.c.) of the different components were used to prepare the mixtures. The activities as represented by the equivalents of sugar formed at the end of half an hour, in each case were as follows.— (1) 0·2 c.c.; (2) 0·3 c.c.; (3) 16·3 c.c.; (4) 0·1 c.c. and (5) 16·7 c.c. of standard thiosulphate, respectively.

In another experiment, diluted fresh enzyme was added to the aged preparation and the activity measured at the end of 24 hours (Table VI).

TABLE VI.

Time in minutes	Activity in c.c. of thiosulphate			
	Fresh	Aged	Fresh + Aged	
			Expected	Found
5	3·1	2·1	2·6	2·4
10	3·5	..	..	..
20	4·4	2·4	3·4	2·9
40	5·4	3·1	4·3	3·3
60	5·6	3·7	4·7	3·9
90	7·1	4·4	5·8	4·5
120	7·7	5·3	6·5	5·5

Thus the activity of the mixture was in every case less than it would have been if the components had functioned independently. The results show that instead of activating the aged enzyme the fresh enzyme was itself partially inactivated as the result of standing in contact with the aged preparation. Since the fresh enzyme would not by itself have lost any of its activity as the result of standing for 24 hours it appears from the above that the aged preparation catalyses the inactivation of the fresh enzyme.

To ascertain whether inactivation of the aged enzyme is due to certain crystalloids present in the aged preparation, specimens of the latter were dialysed at different stages and their activities determined. In no case, however, was the activity appreciably improved as the result of dialysis.

In order to determine whether the electrolytes present in the aged enzyme were responsible for the inactivation of the fresh preparation added, some experiments were conducted repeating the addition of aged enzyme after dialysis. The results were the same as those stated in Table III thereby showing that the electrolytes were not responsible for the inactivation of the fresh enzyme.

Effect of dialysis and age on Taka-diastase.—Taka-diastase (1 g.) was dissolved in 300 c.c. of distilled water and dialysed in collodion bags for 3 days, then made up to 500 c.c., treated with toluene and the activity noted at intervals. The control experiments were with undialysed enzyme. Results showed that both dialysed and undialysed preparations lost their activity readily on standing, the former at a slightly faster rate than the latter. The effect is not, however, so marked as in the case of the malt amylases.

Further work on the mechanism of inactivation is in progress. Effects of various stabilisers on the keeping qualities of enzyme preparations are also being studied.

Summary.

As the result of age, (1) both the liquefying and the saccharifying components of the malt amylases became steadily inactivated, the former at a faster rate than the latter, (2) the optimum P_H is shifted to a less acid reaction and (3) solutions of malt amylases tend to become more acid on standing.

Dialysed malt enzymes are most stable when maintained at their optimum P_H. Undialysed Taka-diastase is best preserved at a less acid reaction than the optimum for its activity. Solutions of Taka-diastase lose their activity rapidly on standing; the effect is more marked in dialysed than with undialysed preparations. Attempts to reactivate aged enzymes have not so far been successful.

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II.—STUDIES IN ENZYME ACTION—PART VI.

HEAT INACTIVATION OF PANCREATIC AMYLASE.

By K. Venkata Giri.

The rate of inactivation of enzymes under varying conditions has been studied by several observers. It has been found in general that the course of the reaction is unimolecular. Tammann (*Z. Physikal. Chem.*, 1895, **18**, 426) showed this to be the case for emulsin, and Pace (*Biochem. J.*, 1930, **24**, 606) obtained similar results with purified trypsin. Pancreatic amylase is an enzyme which loses its activity very rapidly, inactivation occurring at an appreciable rate at room temperatures. It thus forms a very suitable material for examination, in addition to which its importance in physiological processes and industry renders its study very desirable with a view to finding means of retarding as far as possible its spontaneous inactivation. The following experiments were consequently undertaken.

Experimental.

A preparation of pancreatin supplied by Messrs. The British Drug Houses, Ltd., was used in the present investigation. In view of its hygroscopic character, the specimen was dried in a desiccator over anhydrous calcium chloride for at least 24 hours before use. The solutions of pancreatin were made up with conductivity water particularly for experiments in which the kinetics of inactivation had to be followed without the addition of buffers. The starch solution used for the experiments was prepared from the B.D.H., A.R. soluble starch which was washed several times with water, then with alcohol and finally with ether and dried. A 2 per cent. solution of starch was generally used for determining the activity of the enzyme. The reaction was adjusted to P_H 7·1 by adding Sörensen's phosphate buffer. Pure sodium chloride was added to make up a 0·3 per cent. solution of that salt so as to secure the maximum activity of the enzyme under the experimental conditions. Activity was determined according to Sherman and Schlesinger (*J. Amer. Chem. Soc.*, 1915, **37**, 1305).

Effect of concentration on the rate of inactivation of pancreatic amylase.—Preliminary observations having shown that in the initial stage of the reaction the rate of inactivation of pancreatic amylase at 40° conforms to the law for a unimolecular reaction, the effect of varying the concentration was examined. Solutions containing 0·05, 0·10, and 0·16 per cent., respectively, of the solid enzyme were maintained at 40° in a thermostat and the activities determined at frequent intervals. The results are shown in Table I. Curves showing the relation between the logarithm of the activity and time are given in Fig. I.

It will be observed that in the initial stages of the reaction these are approximately straight lines and parallel to each other, showing that the

Fig. I

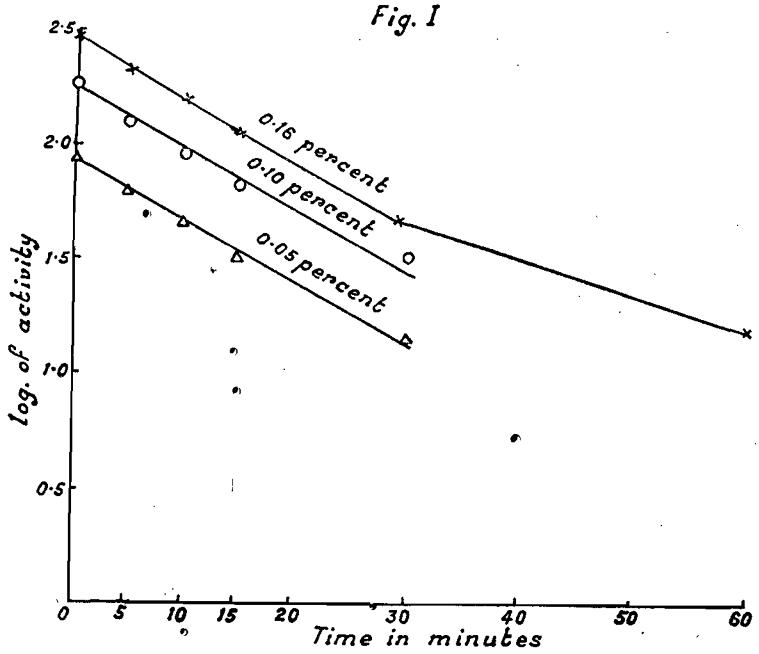
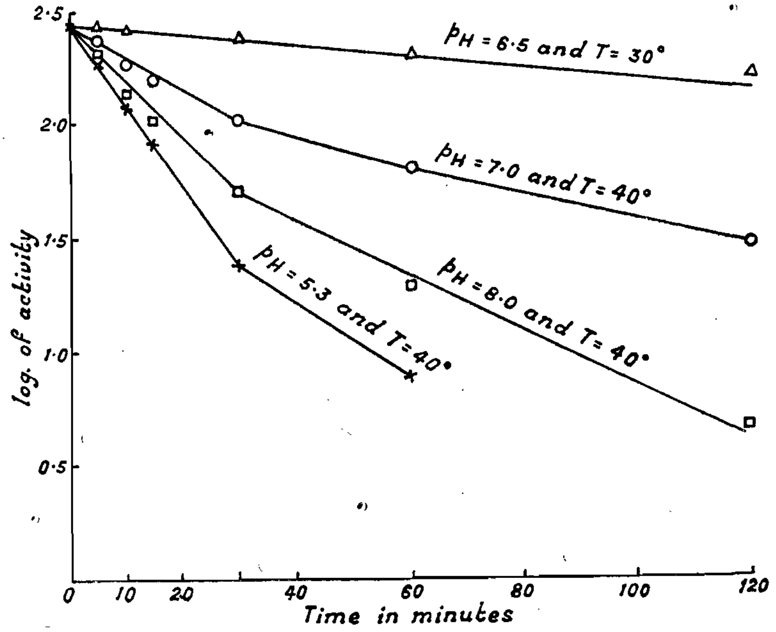

0·16 percent
0·10 percent
0·05 percent
log. of activity
Time in minutes

Fig. II


pH = 6·5 and T = 30°
pH = 7·0 and T = 40°
pH = 8·0 and T = 40°
pH = 5·3 and T = 40°
log. of activity
Time in minutes

reaction velocity is independent of the concentration and that the reaction itself is unimolecular. As the reaction progresses, the rate diminishes, indicating the presence of disturbing factors, or a complexity of the enzyme. As in the case of all enzymes the rate of decomposition varies with P_H, and more definite results were likely to be obtained by using buffered solutions to maintain P_H constant.

TABLE I.

Time in minutes	Activity in mg. of Cu_2O — Concentration per cent. 0·16	0·10	0·05
0	292·0	183·4	87·8
5	220·4	138·0	63·9
10	158·6	95·4	46·8
15	118·0	70·0	33·4
30	51·0	35·0	15·4
60	17·8	..	..
120	6·0	..	..

Effect of P_H *upon the heat inactivation of pancreatic amylase.*—Flasks each containing 80 c.c. of phosphate buffer in varying hydrogen-ion concentration were taken. Pancreatin (0·16 g.) was added to each and sufficient distilled water to make up the volume in each case to 100 c.c. The mixtures were maintained at 40° and the activities determined, the results being shown in Table II.

TABLE II.

Time in minutes	Activity in mg. of Cu_2O — P_H 5·3	5·9	6·5	7·0	7·2	8·0
0	284·1	284·6	283·4	282·0	279·8	285·4
5	192·0	220·8	236·0	238·2	228·4	208·0
10	121·3	163·0	193·0	190·0	182·0	153·0
15	83·9	130·0	160·0	160·0	147·0	112·1
30	25·0	77·4	104·0	108·1	88·2	50·4
60	8·3	41·2	70·2	67·3	56·8	21·0
120	..	11·8	30·2	31·0	25·3	5·0
K	0·0805	0·0504	0·0376	0·0353	0·0411	0·0613

The observations show that the rate of inactivation of the enzyme is the minimum at P_H 6·5–7·0, and increases rapidly as the reaction becomes either more acid or more alkaline. Thus, at P_H 5·3 the activity is only 3 per cent. of its original value at the end of one hour as compared with 25 per cent. at P_H 7·0. This behaviour resembles that of pepsin and catalase studied by Michaelis and Rothstein (*Biochem. Z.*, 1922, **133**, 487) and Morgulis and Befer (*J. Biol. Chem.*, 1928, **77**, 115), respectively.

Fig. II shows the relation between the logarithm of the activity and the time for some of these solutions. The curves resemble those of the unbuffered solutions but appear to exhibit a fairly definite break after about 30 minutes, the initial and final portions being nearly straight. The physical significance is not obvious although the presence of two enzymes is indicated. The simultaneous unimolecular inactivation of two enzymes at different rates would follow an equation of the type $C=ae^{-kt}+be^{-k't}$, but it has not been found possible to fit such an equation to the experimental results. It must be remembered, however, that the expression for the activity is more or less empirical being proportional to the amount of sugar formed under standard conditions in 30 minutes. Since the reaction with starch probably occurs in two stages, liquefaction followed by saccharification, the quantity of sugar is not necessarily proportional to the concentration of the enzymes; but assuming two enzymes one of which acts on the product of the other, the activity is given by the expression

$$la\left\{ t\frac{1}{s}\left(e^{-st}-1\right)\right\},$$

where l is the concentration of the liquefying and s, that of the saccharifying enzyme at any time and t, the time the mixture is allowed to react on the starch. This assumes that l and s remain constant during the reaction which is not the case, and in reality a more complicated expression for the activity is required. The above suffices to show, however, that strictly quantitative results for the decrease in activity can only be obtained when the ratio between the activities of the two enzymes is known and the course of the reaction with starch is followed in detail. Sherman and Schlesinger (*loc.cit.*) have shown that in the case of prolonged action, pancreatin destroys a quantity of starch which is nearly twice the weight of the sugar formed, but this result does not necessarily apply to the initial stages and more work is necessary to elucidate the matter. Unfortunately the experimental difficulties are considerable.

It follows that the present results must be regarded as being only qualitative as far as the kinetics of the reaction are concerned owing to the uncertainty of the method of measuring the activity. At the same time it is probable that for changes in activity which are not large the method gives results which are relatively correct and thus the main conclusion, *viz.*, the inactivation according to a unimolecular equation in the initial stages, the reduction in rate as the reaction proceeds and the effect of hydrogen-ion concentration, may be considered as established.

Influence of temperature on the velocity coefficient.—The experiments described in the foregoing section were repeated at P_H 6·5 and 7·0, which represent the region for optimum stability at 30°. The results are given in table III, and the corresponding graph for P_H 6·5 in Fig. II.

TABLE III.

Time in minutes	Activity in mg. Cu_2O P_H 6.5	7.0
0	282·8	290·0
10	269·2	279·0
15	261·8	272·8
30	244·8	257·0
60	213·8	227·6
120	180·0	204·8
K	0·0048	0·0040

At 40° the rates of decomposition at P_H 6·5 and 7·0 were substantially the same; but at 30° the velocity at P_H 7·0 is distinctly less, and as may be seen from the graph, the reaction followed the unimolecular law throughout the period during which observations were taken.

The energy of activation calculated by means of the formula

$$E = R (\log K_1 - \log K_2) \frac{T_1 T_2}{(T_2 - T_1)}$$

is 39,100 cals. for P_H 6·5 and 41,000 for P_H 7·0.

These results are of the same order as those obtained by Lewis (*Biochem. J.*, 1926, **20**, 965) for the heat denaturation of proteins and by Chick and Martin (*J. Physiol.*, 1910, **40**, 404) for the heat denaturation of oxyhæmoglobin. This suggests that the inactivation of pancreatic amylase may be due to the denaturation of proteins present in the preparation; on the other hand, the marked variation of the rate with the hydrogen-ion concentration of the solution might be ascribed to hydrolysis and the data available afford no indication as to which of these hypotheses is more likely to be correct.

Summary.

A study of conditions relating to heat inactivation of pancreatic amylase shows that solutions of the enzyme are readily inactivated even at the laboratory temperature. The rate of inactivation is at a minimum at P_H 6·5 to 7.0, in the neighbourhood of the optimum reaction for the enzyme.

The course of inactivation follows that of a monomolecular reaction particularly in the early stages. The calculated value of the energy of activation approaches that for protein denaturation. There is evidence to suggest that the heat inactivation of pancreatic amylase is either due to the denaturation of proteins associated with it, or to the hydrolysis of the enzyme itself.

The author's thanks are due to Drs. H. E. Watson and V. Subrahmanyan for their suggestions and criticisms.

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III.—STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS—PART IV.

THE PROTEINS OF FENUGREEK.

(*TRIGONELLA FOENUM GRAECUM*).

By Yelandur Venkamma Sreenivasa Rau and Motnahalli Sreenivasaya.

Fenugreek (*Trigonella fœnum Græcum*) is a leguminous annual, widely cultivated throughout India. Leaves of the young plants are used as green vegetable while the seeds are valued as a condiment and form an essential ingredient of many Indian preparations; they are valued also in Indian medicine for their tonic, astringent and emollient properties. Recently, its medicinal applications have been more widely recognised (Indian Science Congress Abstracts, 1932). On incineration the seeds leave about 7 per cent. of ash which is rich in phosphoric acid (25 per cent. on the weight of the ash). Reutter has noted the presence of several bases in fenugreek such as choline, trigonelline, methylamine, neurine and betain which are derived from the splitting of the lecithin. It is stated that fenugreek can be employed as a substitute for cod-liver oil wherever the latter is prescribed, as in rickets, anæmia and debility. A comprehensive study of the constituents of this seed has been opened by us and this paper relates to the isolation and analysis of two among its proteins.

Experimental.

The sun-dried seeds were ground to flour and passed through a 40-mesh sieve. The soft, snow-white cotyledons constituting the mucilaginous portion separate, and cannot be ground. The flour is light yellow and on analysis gave the following percentages calculated on moisture-free material.

TABLE I.

Ash	Ether extractives	Crude protein N × 6.25	Starch	Cellulose	Lignin	Other Carbohydrates
3·55	8·10	29·90	2·11	21·00	2·23	33·02

Preparation and analysis of the proteins.—Preliminary extraction trials established that a 6 per cent. salt solution removed the maximum quantity of nitrogen representing about 40 per cent. of the total. Distilled water, 6 per cent. saline solution and cold 70 per cent. alcohol, when used consecutively, extract 15, 25, and 5 per cent. respectively, accounting for 45 per cent. of the total nitrogen. The presence of mucilage in the seed renders filtration extremely slow and difficult. Simple gravity filtration yielded clear extracts and was found preferable to centrifuging or pressure filtration.

The flour (500 g.) was mechanically stirred for 2 hours with a 6 per cent. sodium chloride solution (2·5 l.); after filtering through cheese-cloth the residue was again extracted with saline solution (2 l.). Gravity filtration of the extract gave 3 l. of a clear, pale yellow liquid which was dialysed against a

stream of cold distilled water for 5 days until the dialysate was free from chlorides. The precipitated protein (Fraction A) was purified by dissolving in 4 per cent. salt solution followed by a second dialysis; on acidifying the filtrate from this fraction, fraction B was precipitated, and recovered on the centrifuge.

Both preparations were washed several times with distilled water, dehydrated with alcohol and ether, dried, powdered and passed through a 100-mesh sieve. Preparation A (6 g.) was a light, cream-white powder while preparation B (2 g.) was slightly brownish. On analysis, they gave the following percentages:—

TABLE II.

Fraction	Moisture	Ash	Ash and moisture free: Nitrogen	Phosphorus[1]	Sulphur[2]
A	4·74	0·50	17·2	0·00	0·14
B	7·96	0·90	15·91	0·31	0·285

[1] Micro-method of Pregl.
[2] Micro-combustion method of Carius.

The two preparations were analysed by the method of Van Slyke (*J. Biol. Chem.*, 1911, **10**, 15) as modified by Plimmer and Rosedale (*Biochem. J.*, 1925, **19**, 1004), also Plimmer and Lowndes (*Biochem. J.*, 1927, **21**, 247). Arginine was estimated not only in the basic fraction but also separately in the original hydrolysate (Plimmer and Rosedale, *loc. cit.*, 1020). Tyrosine and tryptophan were estimated separately in the original proteins according to the method of Folin and Marenzie (*J. Biol. Chem.*, 1929, **83**, 89). Cystine was estimated by the method of Rimington (*Biochem. J.*, 1930, **24**, 1114). The results are given in tables III and IV (columns 1 and 2), and are compared with globulins of some of the local leguminous seeds (Niyogi, Narayana and Desai, *Ind. J. Med. Res.*, 1931–32, **19**, 1041) expressed as per cent. of total nitrogen.

TABLE III.

Distribution of Nitrogen (per cent. of total).

Form of Nitrogen	Fenugreek A	Fenugreek B	Average of 10 globulins
Melanin	0·6	1·8	1·7
Amide	6·8	7·2	9·7
Basic:			
Arginine	21·2	18·7	16·2
Histidine	18·5	4·7	4·1
Cystine	0·4	0·9	0·6
Lysine	1·9	6·1	8·8
Non-basic:			
Amino	48·9	58·9	55·6
Non-amino	1·9	1·8	3·1
Total ..	100·2	100·1	99·8

The essential amino-acids in the proteins A and B of fenugreek, compared with other globulins, and expressed as percentages of protein (ash and moisture-free), are as follows:—

TABLE IV.

Amino-acid	Fenugreek A	Fenugreek B	Average of 10 globulins
Lysine	1·7	4·9	7·1
Histidine	11·6	2·8	2·3
Arginine (Van Slyke) ..	11·2	9·3	8·0
,, (Direct) ..	12·4	10·5	8·9
Cystine	0·6	1·2	1·9
Tyrosine	5·7	2·1	4·0
Tryptophan	0·5	Present	0·5

Summary.

Two proteins A and B from fenugreek have been isolated and analysed. The globulin (fraction A) is characterised by a surprisingly high content of histidine which is about five times the average amount (2·27 per cent. of protein) contained in the average of ten typical globulins. The excellent and reputed tonic properties of the seed may be partly attributable to this circumstance, histidine being an essential constituent of hæmoglobin. Fraction B is albuminous and is an interesting protein since it appears to contain both phosphorus and sulphur.

Our thanks are due to Mr. N. Narayana for his many helpful suggestions during the course of this work.

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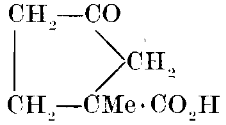
STUDIES IN BRIDGE FORMATION.

PART I. ATTEMPTS TO SYNTHESISE BICYCLIC TERPENE DERIVATIVES BY A NEW METHOD.*

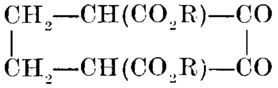
By P. P. Patel and P. C. Guha.

The factors in a synthesis of the bicyclic terpene derivatives camphor, fenchone and others, are the synthesis of *cyclo*pentane derivatives by known methods, and the closing of the *cyclo*hexane ring. Thus Komppa (*Ber.*, 1903, **36**, 4332; *Annalen*, 1909, **368**, 126; *ibid.*, 1909, **370**, 209) and later Perkin and Thorpe (*J.C.S.*, 1906, **89**, 795) synthesised camphoric acid, which through various stages they converted into camphor. Similarly Ruzicka (*Ber.*, 1917, **59**, 1362), starting from laevulinic and bromoacetic ester prepared a *cyclo*pentane derivative,



from which by condensing with another molecule of bromoacetic ester he obtained fenchone. Several similar processes are described in chemical literature, by which *cyclo*pentane derivatives have been utilised for the formation of bicyclic terpene derivatives.

There is, however, a lack of information as to whether it is possible to synthesise such bicyclic compounds by bridging a proper *cyclo*hexane derivative by suitable means. For example, *cyclo*hexane-2 : 3-dione-1 : 4-dicarboxylic ester



(I, R=Me; II, R=Et)

in which there are two hydrogen atoms replaceable by sodium, is expected to give bridged compounds on treatment of its disodium derivative with dihalogen compounds such as acetone dichloride, ethylene chloride, benzal chloride, benzophenone chloride, methylene iodide, carbonyl bromide, etc. In the case of bridging with acetone dichloride, the product was expected to give *apo*camphorquinone on hydrolysing the ester groups and subsequent decarboxylation thus:

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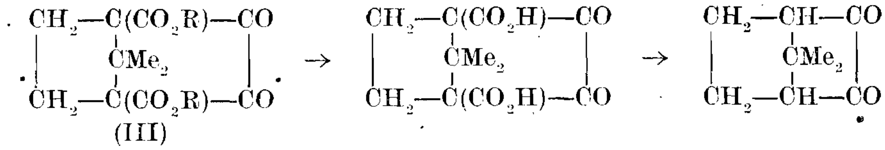
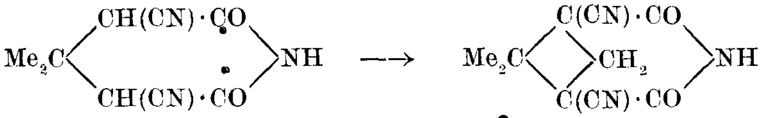
* A preliminary report on this work has been published in the Proceedings of the Indian Science Congress (Chemistry Section, 1930, 177) and was communicated before 15th October 1929.



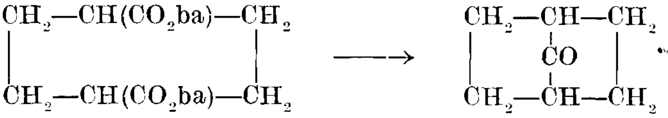
(III)

and with methylene iodide a series of derivatives of norcamphor.

*cyclo*Hexane-2: 3-dione-1: 4-dicarboxylic ester appears to be capable of enolisation in which form it might not lend itself to bridge formation. Recently, however, Kerr (*J. Amer. Chem. Soc.*, 1929, **51**, 614) has succeeded in bridging Guareschi's imide, which is similarly constituted, by treating its disodium derivative with methylene iodide thus:

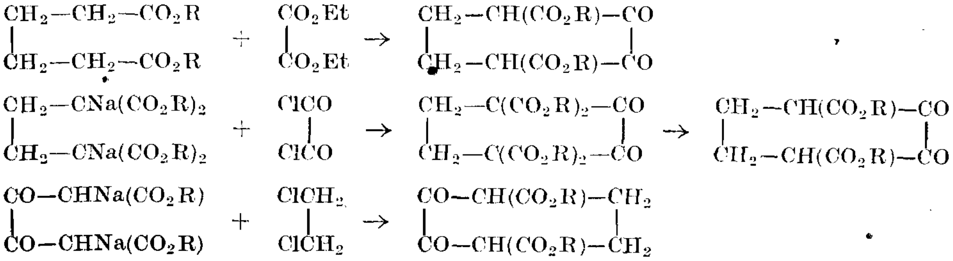


Though, in the case of Guareschi's imide the carbon atoms participating in bridge formation occupy the 1: 3-position, there appears to be no reason why carbon atoms in the 1: 4-position should not also take part in bridge formation and bridging of a *cyclo*hexane derivative in the 1: 4-position has been successful in the hands of Zelinsky (*Ber.*, 1901, **34**, 380), who by heating the barium salt of hexahydroterephthalic acid with calcium carbonate obtained bi*cyclo* (1: 2: 2)-heptanone-7, thus:



The object of the present investigation was based on the above expectations, the experimental verification of which necessitated in the first place, the preparation of compounds resembling ethyl *cyclo*hexane-2 : 3-dione-1: 4-dicarboxylate. Though the corresponding 1: 3- and 1: 4-analogues have been described, the required 1 : 2-diketone is unknown.

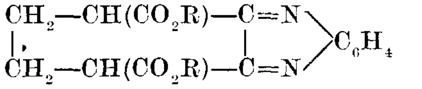
Three methods have been devised for the preparation of the desired diketonic ester: (1) condensation of oxalic ester with adipic ester, (2) interaction of oxalyl chloride with the disodium derivative of $\alpha\alpha\delta\delta$-butanetetracarboxylic ester, and (3) action of ethylene dichloride upon the disodium derivative of ketipic ester thus:



By analogy with the formation of ethyl ketipate from ethyl acetate and ethyl oxalate, Wislicenus and Schwanhausser (*Annalen*, 1897, **297**, 98) attempted to prepare a cyclic diketone by the action of ethyl oxalate upon ethyl adipate, but they could isolate only an open chain compound, *viz.*, ethyl oxalo-adipate. Moreover, it is known that by the action of such dehydrating agents as metallic sodium, sodamide and phosphorus pentoxide, adipic ester is converted into ethyl *cyclo*pentanone-1-monocarboxylate-2.

Though these facts were against the probability of formation of the required diketonic ester, it was hoped that under suitable conditions and with excess of dry sodium alcoholate the desired product might yet be formed. Freshly prepared methyl oxalate on being condensed with dry methyl adipate, employing an excess of dry sodium methoxide as condensing agent, has now according to expectation yielded methyl *cyclo*hexane-2: 3-dione-1: 4-dicarboxylate (I), besides methyl *cyclo*pentanone-1-monocarboxylate-2 and two more oils boiling at different temperatures which appeared in all probability to be dimethyl carbonate and methyl oxalo-adipate respectively, but could not be definitely identified owing to poor yield. The corresponding diethyl ester (II) of *cyclo*hexanedione has been prepared from ethyl adipate. *cyclo*Hexanedionedicarboxylic ester dissolves readily in dilute alkalis, gives a dark violet coloration with ferric chloride and a mono-semicarbazone.

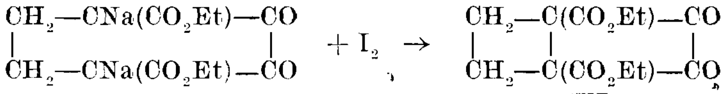
With *o*-phenylenediamine both the methyl and ethyl esters give phenazine derivatives (IV and V).



(IV, R=Me; V, R=Et)

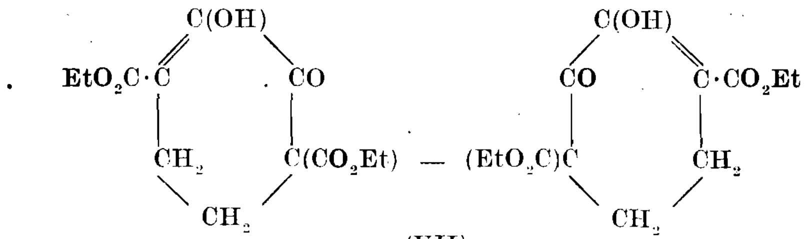
The first experiment on bridge formation was carried out with acetone dichloride by boiling a mixture of the halide, the disodium derivative of (II) and anhydrous benzene continuously, without result. In another attempt, the disodium derivative was heated in a sealed bottle with acetone dichloride in presence of anhydrous xylene, between 135-140° for 6-8 hours, but again the diketo-ester was recovered unchanged. Ethylene chloride, benzal chloride and benzophenone chloride have also been found not to react.

Noting the inertness of the above dihalogen compounds it was thought worth while to study the behaviour of iodine towards the disodium derivative of (II) when the bridged compound (VI) was expected to be formed thus:



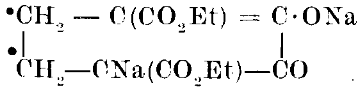
(VI)

The product melted at 90°, gave a deep green coloration with ferric chloride and dissolved readily in dilute alkali, but a compound of formula (VI) cannot behave as an enol; and as it is not the original di-ester the formula (VII) is ascribed to it.



(VII)

Non-formation of the bridged compound (VI) is probably due to the disodium derivative existing in the mono-enolic form (VIII) though the formation of the phenazine derivatives (III, IV) suggests a diketonic structure for the parent di-ester.



(VIII)

Experimental.

Methyl cyclo*hexane*-2 : 3-*dione*-1 : 4-*dicarboxylate* (I).—To dry sodium methoxide obtained from 15 grams of sodium was added dry methyl oxalate (40 g.) and the mixture shaken very vigorously with occasional cooling by immersion in ice-water to prevent too rapid rise of temperature. After addition of anhydrous ether (150 c.c.) an ethereal solution of methyl adipate (12 g.) was slowly added and shaken vigorously for 30 minutes, when the mixture became yellow, and was left overnight at ordinary temperature well protected from moisture. It was then heated in an oil-bath to remove ether followed by dimethyl carbonate at 100°, after which the temperature was maintained at 140° during 12 hours. On acidifying with cold dilute sulphuric acid, a small quantity of thick oil separated which was washed several times with cold water and shaken with a small quantity of cold ethyl alcohol (95 per cent.) liberating a solid crystallising from ethyl alcohol in shining plates m.p. 111-112° (yield about 15 per cent. of theoretical). The diketo-ester is slightly yellowish, gives in alcoholic solution a dark violet coloration with ferric chloride and dissolves readily in cold alkali forming a yellow solution which on acidification loses colour and regenerates the diketo-ester (Found : C, 52·05 ; H, 6·0. $C_{10}H_{12}O_6$ requires C, 52·6 ; H, 5·3 per cent.).

Mono-semicarbazone of the diketo-ester.—An alcoholic solution of the diketo-ester (0·5 g.) was added to a cold saturated solution of semicarbazide hydrochloride and sodium acetate and the mixture thoroughly shaken and cooled in ice for five minutes; the solid product was crystallised from alcohol, m.p. 170° (decomp.) (Found: N, 14·98. $C_{11}H_{15}O_6N_3$ requires N, 14·74 per cent.).

The *phenazine* derivative (IV).—An alcoholic solution of the diketo-ester (0·25 g.), *o*-phenylenediamine (0·15 g.) and sodium acetate (0·3 g.) were heated under reflux for one hour when the solution became orange. On

concentration and cooling a solid separated which crystallised from 95 per cent. alcohol in deep yellow feathery needles, m.p. 175° (Found: N, 9·25. $C_{16}H_{16}O_4N_2$ requires N, 9·33 per cent.).

Methyl *cyclo*pentanone-1-monocarboxylate-2.—The acidic aqueous solution left from the diketo-ester was extracted thrice with ether, the ethereal solution washed successively with dilute sodium carbonate solution and water, and dried. After removing ether, a brown, sweet-smelling oil remained and for the most part distilled under 17 mm. at 106-108°; Bouveault and Locquin (*Compt. rend.*, 1908, **146**, 138) give 105°/19 mm. as the boiling point (Found: C, 58·64; H, 6·53. $C_7H_{10}O_3$ requires C, 59·1; H, 7·00 per cent.).

The *semicarbazone* of methyl *cyclo*pentanone-2-carboxylate was prepared in the usual manner and crystallised from 95 per cent. alcohol, m.p. 176° (Found: N, 20·8. $C_8H_{13}O_3N_3$ requires N, 21·1 per cent.).

Ethyl cyclo*hexane*-2 : 3-*dione*-1 : 4-*dicarboxylate* (II) was prepared as above using ethyl adipate in place of methyl adipate; the resulting oil partially solidified in an ice-chamber during 24 hours. On being washed with a small quantity of cold dilute alcohol pale yellow shining plates, m.p. 56°, were obtained (Found: C. 56·51; H, 6·91. $C_{12}H_{16}O_6$ requires C, 56·25; H, 6·25 per cent.).

The *phenazine* derivative (V) crystallised from alcohol in orange coloured needles, m.p. 101° (Found: N, 8·81. $C_{18}H_{20}O_4N_2$ requires N, 8·5 per cent.).

Disodium derivative (VIII) of the diketo-ester (II).—An anhydrous ethereal solution of the diketo-ester (11 g.) was added to an absolute alcoholic solution of sodium (2 g.) and the yellow powdery product washed with anhydrous ether (Found: Na, 14·9. $C_{12}H_{14}O_6Na_2$ requires Na, 15·3 per cent.).

Chloracetol (acetone dichloride), prepared according to the method of Friedel and Landenberg (*Annalen*, 1867, **142**, 315), could be obtained pure only after a large number of fractional distillations, and boiled at 68–70°/686 mm.

Attempts to prepare compound (V) *by bridging the diketo-ester* (II) *with chloracetol.*—The disodium derivative (7 g.), anhydrous benzene (50 c.c.) and chloracetol (7 g.) were heated together in absence of moisture on a water-bath under reflux for 8–9 hours, when the red solution was alkaline. The thick red semi-solid left after removing benzene by distillation was dissolved in cold water and extracted with ether, the residue from which on distillation was negligible. The aqueous solution on being acidified with dilute sulphuric acid gave an oil which on cooling partly solidified; when crystallised from 93 per cent. alcohol the product melted at 55–56° and was identified with the original di-ester (II). The same experiment conducted in presence of xylene at 135–140° in a sealed bottle gave no better results.

Action of iodine upon the disodium derivative of (II): *Formation of compound* (VII).—To an alcoholic suspension of the disodium derivative

obtained from the diketo-ester (5·5 g.) and sodium (1 g.) was added portionwise a solution of iodine (2·5 g.) in absolute alcohol; after two hours on a water-bath the residue from alcohol gave on neutralisation a thick brown oil which in contact overnight with a small quantity of 93 per cent. alcohol yielded a solid. This crystallised readily from alcohol, m.p. 90°; it is soluble in alkali and gives a deep green coloration with ferric chloride (Found: C, 56·08; H, 5·31. $C_{24}H_{30}O_{12}$ requires C, 56·47; H, 5·88 per cent.).

The action of different hydrolysing agents, methylene iodide, and di-acid halides upon the di-ester (I) is under investigation.

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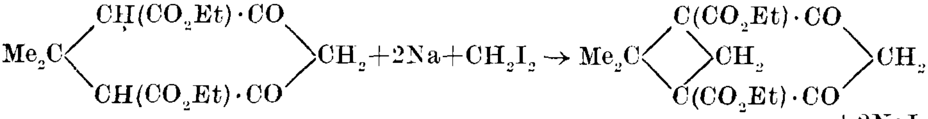
STUDIES IN BRIDGE FORMATION.

PART II. FORMATION AND STABILITY OF A BRIDGED *CYCLO*HEXENONE, 1-DIMETHYLETHANE-$^{1:2:2}$-III-$^{1:4:4}$-*CYCLO*BUTANE-2-ONE.*

By P. S. Mayuranathan and P. C. Guha.

Although the synthesis of carone, fenchone and camphor has long been realised, only one attempted synthesis of nopinone has been recorded (Simonsen and Perkin, *J.C.S.*, 1907, **91**, 1736). Analogous with the synthesis of carone by Baeyer (*Ber.*, 1891, **27**, 1919; 1896, **29**, 6) from dihydrocarvone hydrobromide by treatment with potash, Simonsen and Perkin tried to effect the synthesis of nopinone with the hydrobromide of 4-*iso*propylidene-*cyclo*hexanone.

Kerr discovered recently (*J. Amer. Chem. Soc.*, 1929, **51**, 615) the formation of a synthetic bridged ring from the Gaureschi imide and methylene iodide. A similar study was contemplated with Scheiber and Miesel's ester (*Ber.*, 1915, **48**, 238), *viz.*, ethyl *cyclo*hexane-1: 1-dimethyl-3: 5-diketo-2: 6-dicarboxylate (I) expecting a synthetic bridged carbon ring of the type (II), which was to provide an easy step to the synthesis of the dicyclic ketone, nopinone.



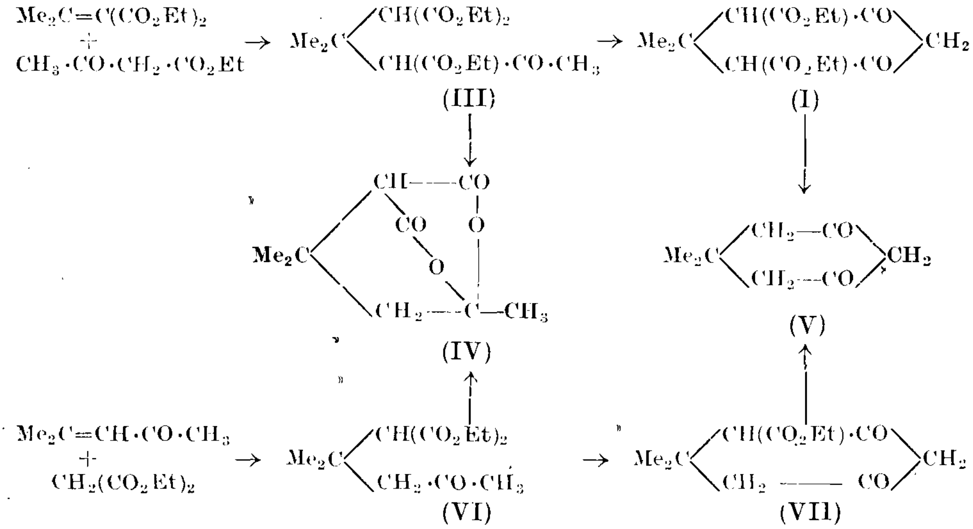
(I) (II) +2NaI

Although the di-ester does not react with methylene iodide to yield the desired bridged product (II), study of the constitution of the starting material by hydrolysis has led to the discovery of a new dicyclic ketone, $C_8H_{10}O$. Applying the nomenclature of Beesley and Thorpe (*J.C.S.*, 1920, **117**, 591) it may be distinguished as 1-dimethylethane $^{1:2:2}$-III-$^{1:4:4}$-*cyclo*butane-2-*one*. In future, for simplicity, this ketone will be referred to as the bridged *cyclo*-hexenone (X).

Scheiber and Miesel (*loc. cit.*) state that *iso*propylidenemalonic ester and acetoacetic ester condense to give ethyl *cyclo*hexane-1: 1-dimethyl-3: 5-diketo-2: 6-dicarboxylate (I), in presence of atomic proportion of sodium. Proof of ring structure was afforded by its conversion on hydrolysis into dimethyl-dihydroresorcin (V). We find that in presence of traces of sodium ethylate simple addition takes place producing a substituted malonic ester (III), which gives on hydrolysis a dilactone (IV), previously obtained by Vorländer

* Preliminary reports on this work were published in the Proceedings of the Indian Science Congress (Chemistry Section, 1931, 159; 1932, 117) and were communicated before 15th October, 1930 and 1931, respectively.

(*Annalen*, 1896, **194**, 257) and recently by Khuda (*J.C.S.*, 1929, 201) from the addition product (VI) of mesityl oxide and malonic ester.

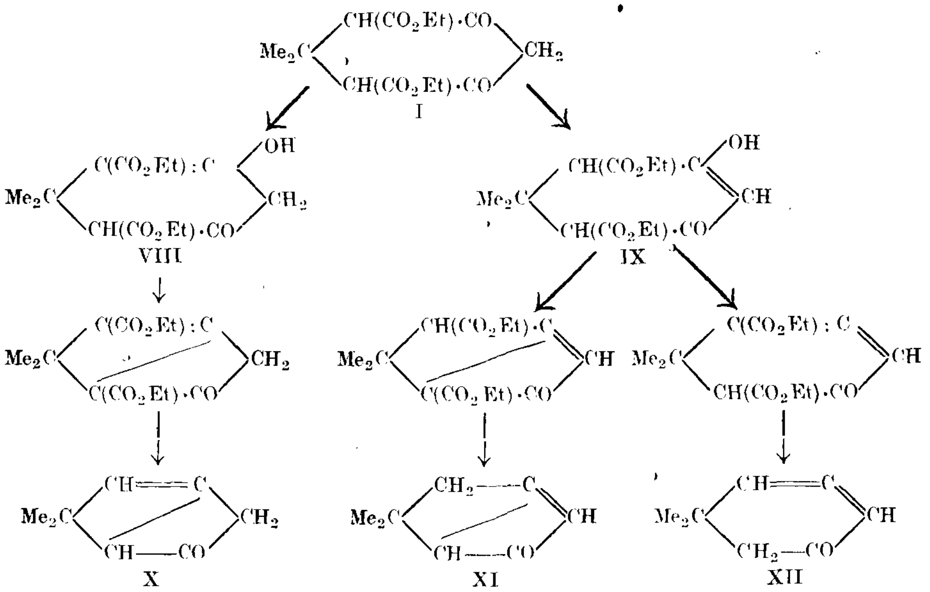


Although the open-chain ester (III) was not actually isolated by Scheiber and Miesel they assumed its intermediate formation and conversion on distillation or on treatment with semicarbazide acetate into the ring-closed ester (I). It has now been found that the open-chain ester is not transformed into the ring-closed ester under any of the conditions mentioned above. Conversion of (III) into (I) and (IV), which are compounds of established constitution, supports the structure ascribed to (III).

As mentioned before, the ring-closed ester (I) on hydrolysis gives besides dimethyldihydroresorcin the bridged *cyclo*hexenone (X). The condition controlling the exclusive production of the bridged *cyclo*hexenone has now been found to depend on the quantity rather than on the quality of the hydrolysing agent. The bridged *cyclo*hexenone is not acidic. Unlike dimethyldihydroresorcin (V) and its carboxylic esters (I and VII) it does not give colour with ferric chloride, proving its incapacity to enolise. It decolourises an acetic acid solution of bromine and an acetone solution of permanganate, manifesting unsaturation. It corresponds with an empirical formula $C_8H_{10}O$ and gives a semicarbazone. It has a molecular weight of 126 in glacial acetic acid solution thus agreeing with the monomolecular formula (calc. 122) : in benzene, however, the value is almost double (246) showing that the molecules are associated in this solvent. Scheiber and Miesel failed to isolate this interesting ketone, as they hydrolysed the ester to the resorcin with excess of strong baryta, which we find converts the ketone into dimethyldihydroresorcin. The fact that neither Vorländer's mono-ester (VII) (*loc. cit.*) nor dimethyldihydroresorcin gives the bridged *cyclo*hexenone on dehydration proves definitely that the two ester groups in propinquity with the *gem*dimethyl group are responsible for this interesting reaction. The process can be explained by assuming an initial enolisation, followed by loss of one molecule of water from the hydroxyl

with a reactive hydrogen atom in the *para*-position. Moreover, enolisation and subsequent dehydration must be assumed to have preceded decarboxylation, as otherwise the entire formation of the bridged *cyclo*hexenone to the exclusion of dimethyldihydroresorcin cannot be explained. If decarboxylation had preceded dehydration, dimethyldihydroresorcin should have been the intermediary, but as mentioned before this cannot be converted into the bridged *cyclo*hexenone. The presence of the two ester groups alone, without the *gem*dialkyl group in the resorcin residue, is insufficient for bridge formation as evidenced by the failure of the phenyl analogue of ester (I), *viz.*, ethyl *cyclo*hexane-1-phenyl-3: 5-diketo-2: 6-dicarboxylate, to give a corresponding bridged *cyclo*hexenone.

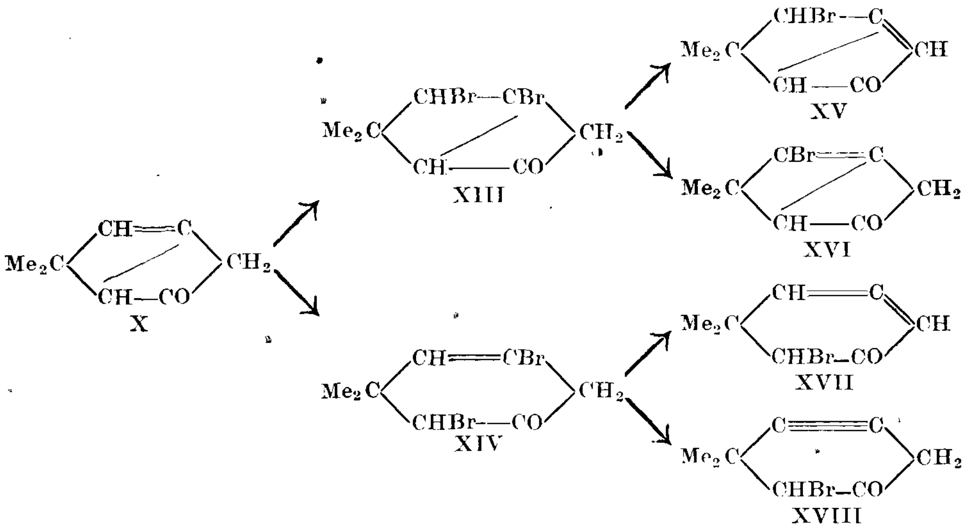
At this stage the following three possible formulæ (X, XI and XII) for the bridged *cyclo*hexenone may be considered:



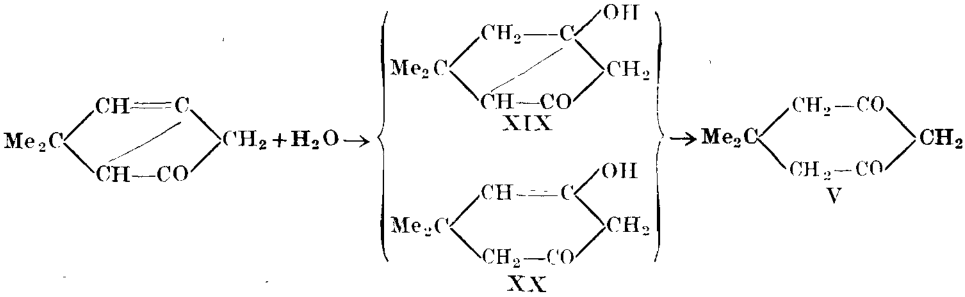
Structure (XII) can be rejected as its existence is contrary to the fundamental conceptions of strain hypothesis. The fact that Vorländer's ester (VII), which to give the bridged *cyclo*hexenone must enolise only as in the phase (IX), does not undergo this type of dehydration indicates the phase (VIII) as more probable: presence of two ester groups in the position adjacent to the dimethylmethylene group thus appears to be indispensable for the reaction. Also the ketonic property of the compound excludes internal ether formation in dimethyldihydroresorcin. Nevertheless, before finally accepting structure (X) it should be stated that there are circumstances in pinene and camphor chemistry to question its stability, though they bear no direct analogy with the present case. It may be mentioned that Ingold succeeded in effecting the synthesis of a similar bridged ring (*J.C.S.*, 1921, **121**, 1143) in his attempt to establish the presence of a Dewar phase for orcinol, but he discovered that as soon as formed the compound was

rearranged to the Kekulé phase. It is remarkable that the new bridged *cyclo*hexenone does not undergo a similar transformation, and its stability must be ascribed to the influence of the *gem*dimethyl group.

The bridged *cyclo*hexenone takes up two atoms of bromine to give a dibromo-compound which remains unchanged with excess of the halogen. If it is assumed that the bromine is added at the bridged bond instead of the double bond, two more atoms of bromine might readily have been added to yield a tetrabromo-compound. Moreover, removal of hydrogen bromide leads to a monobromo-compound (XV or XVI), either formula for which is more acceptable than the alternative structures (XVII and XVIII):



Probably also addition of water to the bridged *cyclo*hexenone must occur at the double bond giving an unstable intermediate compound (XIX) which later would rearrange itself to dimethyldihydroresorcin (V).



The bridged *cyclo*hexenone resists mild oxidation, and with a boiling solution of alkaline permanganate or cold hypochlorite gives $\beta\beta$-dimethylglutaric acid besides other acids not isolated. In this connection the oxidation of Perkin and Thorpe's di*cyclo*pentane acid (*J.C.S.*, 1901, **79**, 729) by Farmer and Ingold (*J.C.S.*, 1922, **121**, 128) may be mentioned in which an initial breakdown of the bridged bond by addition of water was supposed, though

subsequently rejected. Probably, the bridged *cyclo*hexenone is first transformed into the resorcin which then undergoes oxidation to $\beta\beta$-dimethylglutaric acid.

Experimental.

Addition of acetoacetic ester to iso*propylidenemalonic ester: Formation of the ester* (III).—*iso*Propylidenemalonic ester (200 g.) and acetoacetic ester (130 g.) were added to a one per cent. solution of sodium ethoxide (300 c.c.). The mixture was heated in a sealed bottle under pressure at 105-110° for 60 hours, and after removing excess of alcohol was acidified with 5 per cent. sulphuric acid and extracted with ether; this was dehydrated by anhydrous magnesium sulphate and removed, when the ester distilled under 5 mm. pressure at 155-158° as a light yellow oil. Yield 30 per cent. (Found: C, 57·8; H, 8·5. $C_{16}H_{26}O_7$ requires C, 58·2; H, 8·0 per cent.).

Hydrolysis of the ester (III) : *Formation of the dilactone* (IV).—The ester (5 g.) was mixed with concentrated hydrochloric acid (25 c.c.) and heated under reflux for 12 hours, the resulting white solid being crystallised from benzene (m.p. 136°; yield, 0·8 g.). Its identity with Vorländer's dilactone was established by mixed melting point (Found: C, 58·5; H, 6·8. $C_9H_{12}O_4$ requires C, 58·7; H, 6·5 per cent.).

Ethyl cyclo*hexane*-1 : 1-*dimethyl*-3 ; 5-*diketo*-2 : 6-*dicarboxylate* (I).—A mixture of *iso*propylidenemalonic ester (200 g.), acetoacetic ester (130 g.) and sodium (23 g.) in alcohol (300 c.c.) was boiled for 24 hours on the water-bath. Excess of alcohol was removed, the yellow sodium derivative decomposed with 5 per cent. sulphuric acid and the oil that separated extracted with ether. The crude ester was dissolved in 5 per cent. sodium carbonate, ether extracted and the aqueous layer acidified with 2*N* sulphuric acid. The ester was taken up in ether, dried and the ether removed, the residual brown oil distilling at 190–195° under 4–5 mm. as a thick yellow oil. Yield, 85 per cent. (Found: C, 59·3; H, 7·5. $C_{14}H_{20}O_6$ requires C, 59·2; H, 7·1 per cent.).

Ring-closure of the additive ester (III) *to compound* (I).—The ester (15 g.) was added to alcohol (25 c.c.) containing sodium (1 g.) and the mixture heated on a water-bath for about 12 hours, 10 grams of the ester (I) being obtained. The semicarbazone was a white powder from alcohol, m.p. 205° (decomp.) (Found: C, 48; H, 6·6; N, 21·1. $C_{16}H_{26}O_6N_6$ requires C, 48·2; H, 6·5; N, 21·1.).

Hydrolysis of the ester (I) *with baryta: Formation of dimethyldihydroresorcin* (V).—The ester (10 g.) was hydrolysed with concentrated baryta (100 c.c.) for a day. The liquid was concentrated, and the dried ether extract on being freed from ether left a solid residue (3 g.) which crystallised from benzene in shining yellow needles, m.p. 148°, identical with Vorländer's dimethyldihydroresorcin.

1-*Dimethylethane*-$^{1:2:2}$ III $^{1:4:4}$-cyclo*butane*-2-*one*.

Hydrolysis of ester (I) *with* (a) *hot and cold ethyl alcoholic potash: Formation of the bridged* cyclo*hexenone* (X).—The ester (10 g.) was mixed with 25 per cent.

ethyl alcoholic potash (25 c.c.) and kept for 2 days at room-temperature or heated under reflux for 6 hours. After removing excess of alcohol under suction the residue was acidified with 5*N* hydrochloric acid, effervescence being due to decomposition of the sodium carbonate formed on hydrolysis. The solution was saturated with ammonium sulphate and extracted half a dozen times with ether, which when washed and evaporated left a crystalline white solid separating from ordinary alcohol as long rhombic plates m.p. 176° (corr.); yield, 3 grams. The same product was obtained using methyl alcoholic potash.

Hydrolysis with (b) 75 *per cent. sulphuric acid or concentrated hydrochloric acid.*—The ester (20 g.) was mixed with 75 per cent. sulphuric acid (50 c.c.) or with concentrated hydrochloric acid (50 c.c.), and both heated for 6 hours on a water-bath. The mixtures were diluted with 50 c.c. of water, saturated with ammonium sulphate and ether extracted. On purification the compound as in (*a*) was obtained. Yield, 6 and 8 grams respectively (Micro, Found: C, 78·5, 78·2; H, 8·1, 8·5. $C_8H_{10}O$ requires C, 78·7; H, 8·2 per cent. Mol. wt. in benzene, 246; camphor, 261; formic acid, 286; acetic acid, 126. $C_8H_{10}O$ requires 122.).

The semicarbazone of (X) was obtained from alcohol as a white powder m.p. 220° (decomp.) (Found: C, 60·1: H, 7·5; N, 23·4. $C_9H_{13}ON_3$ requires C, 60·4; H, 7·3; N, 23·4 per cent.).

Hydrolysis of Vorländer's ester (VII) *to dimethyldihydroresorcin by* (a) *cold alcoholic potash.*—The ester (5 g.) was added to ethyl alcoholic potash (12 c.c. of 25 per cent.) and kept for 2 days at room temperature; excess of alcohol was removed under diminished pressure and the residue acidified with 5*N* sulphuric acid which liberated carbon dioxide. The acid solution was saturated with ammonium sulphate, ether extracted half a dozen times, dried and freed from ether, the residual yellow solid crystallising from benzene in yellow needles, m.p. 148°.

Hydrolysis with (b) *baryta.*—The ester (5 g.) was boiled under reflux with a strong solution of baryta (200 c.c.), the solution concentrated, acidified with strong hydrochloric acid and ether extracted. The extract dried with anhydrous magnesium sulphate left a solid which crystallised from benzene in yellow needles m.p. 148°, identical with the product from (*a*) and with dimethyldihydroresorcin.

Addition of water to the bridged cyclo*hexenone* (X) *with baryta.*—The compound (5 g.) was heated with concentrated baryta (100 c.c.) under reflux for 6 hours, when the solution was evaporated, acidified with concentrated hydrochloric acid and extracted with ether; the residual solid on crystallisation from benzene was obtained as fine yellow needles m.p. 148°, identified with dimethyldihydroresorcin.

The *dibromide* of the bridged *cyclo*hexenone (XIII).—The ketone (10 g.) was dissolved in glacial acetic acid (50 c.c.) and treated with bromine (4·5 c.c.) the colour fading gradually. Excess of acid was evaporated and the brown solid crystallised from a large quantity of absolute alcohol, which deposited a

brown powder m.p. 196°. Yield, 10·6 grams (Found: C, 34·0; H, 3·72; Br, 56·3. $C_8H_{10}OBr_2$ requires C, 34·0: H. 3·54: Br. 56·7 per cent.).

The *monobromo*-derivative of the bridged *cyclo*hexenone (XV or XVI).—The dibromo-compound (2 g.) was added to methyl alcohol (20 c.c.) containing (0·8 g.) potassium hydroxide and heated under reflux during 6 hours; after evaporating alcohol the product was crystallised from alcohol in pale brown needles m.p. 142°. Yield, 0·8 g. (Found: C, 47·5: H, 4·6; Br, 39·5. C_8H_9OBr requires C, 47·7; H, 4·5; Br, 39·8).

Oxidation of the bridged cyclo*hexenone* (X) *with* (a) *permanganate.*—The ketone (5 g.) was suspended in boiling 2*N* sodium carbonate (1 *l.*) and treated with potassium permanganate (10 g.) during 6 hours. Manganese dioxide was filtered, the solution decolorised with sulphur dioxide, evaporated to small bulk, acidified with 40 per cent. sulphuric acid and ether extracted half a dozen times. The residue was redissolved in 2*N* sodium hydroxide, filtered from unchanged di*cyclo*hexenone, acidified and again ether extracted. The thick oil (3 g.) thus obtained became a crystalline mass on standing for a month, recrystallising from benzene in plates, m.p. 101°.

Oxidation with (b) *hypochlorite.*—Into a 30 per cent. ice-cooled solution of sodium hydroxide (100 c.c.) was passed chlorine generated from potassium permanganate (2 g.) and concentrated hydrochloric acid (20 c.c.) and the ketone (5 g.) was added. The mixture was shaken constantly for 12 hours and then treated with sodium bisulphite till a sample of it liberated no more chlorine with hydrochloric acid. The solution was evaporated to small bulk and ether extracted several times. The unchanged product was removed as in the previous case and the residual oil partially solidified after a fortnight. The separated solid crystallised from benzene m.p. 101° and was identified along with the acid obtained in (*a*) as ββ-dimethylglutaric acid (Found: C, 52·1; H, 8; Equiv., 80. $C_7H_{12}O_4$ requires C, 52·5; H, 7·5; Equiv., 80).

Attempt to bridge ester (I) *with methylene iodide.*—The ester (35 g.) was added to alcohol (80 c.c.) in which sodium (6 g.) had been dissolved, methylene iodide (40 g.) was added and after 12 hours the mixture was heated under reflux in an oil-bath at 105-110° for 12 hours. Excess of alcohol was removed under reduced pressure and the residue acidified with 2*N* sulphuric acid and the separated oil extracted with ether: this was dried by anhydrous magnesium sulphate and removed, when a thick brown oil remained. On distillation under 2 mm. a solid began to deposit in the condenser at about 170–180°; it crystallised readily from benzene, m.p. 148° and was identified as dimethyldihydroresorcin.

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